26th Fungal Genetics Conference at Asilomar

March 15-20
2011

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Brief Schedule

DAY	MORNING	AFTERNOON	EVENING
Tuesday, March 15	Satellite meetings	Arrival Registration	Dinner Mixer
Wednesday, March 16	Plenary Session I **Genome Evolution**	Concurrent Sessions I	Poster Session I
Thursday, March 17	Plenary Session II **Symbioses**	Concurrent Sessions II	Poster Session II
Friday, March 18	Plenary Session III **Growth and Reproduction**	Concurrent Sessions III	Poster Session III
Saturday, March 19	Plenary Session IV **Regulatory Networks**	Concurrent Sessions IV	Banquet and Perkins/Metzenberg Lecture Party
Sunday, March 20		Lunch Departure	

Citations

The program book form teh 26th Fungal Genetics Conference is published as a supplement to the Fungal Genetics Reports. Abstracts will beavailable on the FGSC website and may be cited as follows: Fungal Genetics Reports 58(Suppl): #

Posters

Please set up your poster in the garage below Fred Farr Forum immediately after lunch the day of your poster session. Posters will be available to view beginning as they are set up following lunch. The size of the poster should not exceed 4 feet x 4 feet. Two authors will share a 4 x 8 poster stand. Authors of ODD numbered posters should be at their poster from 7:30- 8:30 and authors of EVEN numbered posters should be at their posters from 8:30 - 9:30. If you have a poster that is not in the program book, you may post it at a space where the poster was listed as "Withdrawn."

Topic	# Range	Posters I	Posters II	Posters III
Comparative and Functional Genomics	1 - 112		1 - 112	
Gene Regulation	113- 223	113 - 223		
Cell Biology	224- 321		224 - 321	
Biochemistry and Metabolism	322- 388	322 - 388		
Population and Evolutionary Genetics	389- 445			389 - 445
Pathogenic and Symbiotic Interactions	446- 601			446 - 601
Education	602- 606			602 - 606
Other Topics	607- 678	607 - 653	654 - 670	671 - 678

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

TWENTY SIXTH FUNGAL GENETICS CONFERENCE

SCIENTIFIC PROGRAM

Tuesday, March 15

3:00 pm – 10:00 pm	Registration	**Administration Building**
6:00	Dinner	**Crocker Hall**
7:30 pm – 10:30 pm	Social Reception (Mixer)	**Merrill Hall**

Wednesday, March 16

7:30 am - 1:00 pm	Registration	**Administration**
7:30 am - 8:30 am	Breakfast	**Crocker Hall**

8:30 am – 12:00 pm **Plenary Session I** **Merrill Hall and Chapel**

Genome Evolution Chair: Ralph Dean

Jeff Townsend	**Comparative transcriptomics and development of perithecia**
David Hibbett	**Deep Rot: Phylogenetic and comparative genomics perspectives on the evolution of the wood decay apparatus in Agaricomycotina**
Antonis Rokas	**The birth, evolution and death of metabolic pathways in fungi**
Geraldine Butler	**Evolution of hypoxic regulation in Candida species**
Martijn Rep	**Genome dynamics in the *Fusarium oxysporum* species complex**

12:00- 1:00 pm Lunch **Crocker Hall**
Box lunches will be available on a first come, first served basis for meeting attendees on the deck outside of the Administration Building.

Following lunch, the mornings speakers will be available on the benches outside the administration building to meet with students. Please allow time for students to meet the speakers. In the event of rain, please go inside the administration building.

12:15 pm – 1:30 pm**, Ad hoc workshops** (Please do not take box lunches from ad hoc sessions unless you are attending the session)

Neurospora Lunch/Policy Committee	**Chapel**
Magnaporthe Comparative Genomics (until 1:45 pm)	**Fred Farr Forum**

3:00 pm – 6:00 pm **Concurrent Sessions I**

Comparative and Functional Genomics	Jason Stajich and Dawn-Anne Thompson	**Merrill Hall**
Photobiology	Alex Idnurm and Luis Corrochano	**Chapel**
Fungal Effectors	Mark Glijzen and Bart Thomma	**Heather**
Symbiosis	Alga Zuccaro and Sebastien Duplessis	**Fred Farr Forum**
Evolution of Sex in Fungi	Paul Dyer and Robert Debuchy	**Kiln**
Evolution of Centromeres	Kaustuv Sanyal and Judy Berman	**Nautilus**

6:00 Dinner, **Crocker Hall**

7:30 pm – 10:30 pm **Poster Session I** **Fred Farr Forum Garage**

Poster Number	Topics
113 - 223	Gene Regulation
322 - 388	Biochemistry and Metabolism
607 - 653	Other Topics

Authors of ODD numbered posters should be at their poster from 7:30- 8:30 and authors of EVEN numbered posters should be at their posters from 8:30 - 9:30.

Thursday, March 17

7:30 am - 1:00 pm Registration **Administration**
7:30 am - 8:30 am Breakfast **Crocker Hall**

8:30 am – 12:00 pm **Plenary Session II** **Merrill Hall and Chapel**

Symbioses Chair: Francis Martin

Regine Kahmann	**Effectors and the establishment of biotrophy in smut fungi**
Natalia Requena	**Root sweet home: signaling in the arbuscular mycorrhizal symbiosis**
Duur Aanen	**Artificial selection of Termitomyces fungi by termites**
Christian Hertweck	**Endofungal bacteria as producers of mycotoxins**
Elizabeth Arnold	**Evolutionary origins of endophytic fungi**

12:00- 1:00 pm Lunch **Crocker Hall**

Box lunches will be available on a first come, first served basis for meeting attendees on the deck outside of the Administration Building.

Following lunch, the mornings speakers will be available on the benches outside the administration building to meet with students. Please allow time for students to meet the speakers. In the event of rain, please go inside the administration building.

12:15 pm – 2:00 pm**, Ad hoc workshops** (Please do not take box lunches from ad hoc sessions unless you are attending the session)

Career Luncheon **Seascape Dining Room**
Trainees are welcome to seek advice from career scientists at the Career Luncheon. Career mentor tables will be located in the Seascape Dining Room of Crocker Hall, and will arranged by mentoring topic. Table-hopping is encouraged.

JGI Fungal Genomics Program:Tools and Applications **Merrill Hall**

3:00 pm – 6:00 pm **Concurrent Sessions II**

Fungus-Host Signaling	Barbara Howlett and Gunther Döhlemann	**Merrill**
Interactions between Fungi and Prokaryotes	Teresa Pawlowska and Paola Bonfante	**Kiln**
Emergent Fungal Diseases	John Taylor and Matthew Fisher	**Chapel**
Regulation of Septation During Growth and Development	Oded Yarden and Stephan Seiler	**Heather**
Secretion, Endocytosis and Membrane Trafficking	Brian Shaw and Miguel Peñalva	**Fred Farr Forum**
ChIP-chip/ChIP-seq: protein interactions with DNA	Tom Michell and Michael Freitag	**Nautilus**
Education and Public Outreach	Pat Pukkila and Mimi Zolan	**Scripps**

6:00 Dinner **Crocker Hall**

7:30 pm – 10:30 pm **Poster Session II** **Fred Farr Forum Garage**

Poster Number	Topics
1 - 112	Comparative and Functional Genomics
224- 321	Cell Biology
654 - 670	Other Topics

Authors of ODD numbered posters should be at their poster from 7:30- 8:30 and authors of EVEN numbered posters should be at their posters from 8:30 - 9:30.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Friday, March 18

7:30 am - 1:00 pm Registration **Administration**
7:30 am - 8:30 am Breakfast **Crocker Hall**

8:30 am – 12:00 pm **Plenary Session III** **Merrill Hall and Chapel**

Growth and Reproduction Chair: Steve Harris

Jeff Rollins	**The developmental determinacy and reprogramming of *Sclerotinia sclerotiorum* apothecia**
Amy Gladfelter	**Nuclear anarchy and cortical order in *Ashbya gossypii***
Jose Perez-Martin	**Connections between cell cycle, morphogenesis and pathogenicity in fungi: the case of *Ustilago maydis***
Stephan Seiler	**Dissecting COT1 NDR kinase regulation and signaling in *Neurospora crassa***
Hanna Johannesson	**Longstanding evolutionary puzzles: Fungi as holders of the missing pieces**

12:00- 1:00 pm Lunch **Crocker Hall**
Box lunches will be available on a first come, first served basis for meeting attendees on the deck outside of the Administration Building.

Following lunch, the mornings speakers will be available on the benches outside the administration building to meet with students. Please allow time for students to meet the speakers. In the event of rain, please go inside the administration building.

Ad hoc workshops (Please do not take box lunches from ad hoc sessions unless you are attending the session)

12:15 pm – 1:45 pm
Colletotrichum Workshop **Scripps**

12:15 pm - 1:30 pm
Fungal Genome Tools **Chapel**

3:00 pm – 6:00 pm **Concurrent Sessions III**

Cell Cycle, Development, and Morphology	Nick Talbot and Meritxell Riquelme	**Fred Farr Forum**
Host Selective Toxins	B. Gillian Turgeon and Tim Friesen	**Nautilus**
Fungicides and Antifungals	Leah Cowen and Sabine Fillinger	**Chapel**
Biobased products, Biofuels, and Bioenergy	Jonathan Walton and Peter Punt	**Merrill**
High Throughput Methods for Filamentous Fungi	Marco Berg-van-den and Aric Wiest	**Heather**
Population Genomics	Jim Anderson and Eva Stuckenbrock	**Kiln**
Dimorphic Transitions in Fungi	Julia Kohler and Anne Dranganis	**Scripps**

6:00 Dinner **Crocker Hall**

7:30 pm – 10:30 pm **Poster Session III** **Fred Farr Forum Garage**

Poster Number	Topics
389 - 445	Population and Evolutionary Genetics
446 - 601	Pathogenic and Symbiotic Interactions
602 - 606	Education
671 - 678	Other Topics

Authors of ODD numbered posters should be at their poster from 7:30- 8:30 and authors of EVEN numbered posters should be at their posters from 8:30 - 9:30.

Saturday, March 19

7:30 am - 1:00 pm Registration **Administration**
7:30 am - 8:30 am Breakfast **Crocker Hall**

8:30 am – 12:00 pm **Plenary Session IV** **Merrill Hall and Chapel**

Regulatory Networks Chair: Nancy Keller

Brenda Andrews	**Mapping cellular pathways using yeast functional genomics**
Dawn Anne Thompson	**It's About Time: Evolutionary rewiring of regulatory networks**
Brett Tyler	**How fungal and oomycete proteins enter plant and animal cells**
Jay Dunlap	**Genetic and genomic approaches to light and clock regulation of development**
John Linz	**Regulation of the compartmentalization of secondary metabolite biosynthesis**

12:00- 1:00 pm Lunch **Crocker Hall**

Following lunch, the mornings speakers will be available on the benches outside the administration building to meet with students. Please allow time for students to meet the speakers. In the event of rain, please go inside the administration building.

2:00 pm – 5:00 pm **Concurrent Sessions IV**

Genome Defense Mechanisms, Epigenetics and RNAi	Rodolfo Aramayo and Yi Liu	**Merrill Hall**
Systems and Synthetic Biology	Han de Winde and Merja Pentilla	**Fred Farr Forum**
Fungi that Infect Humans	Aaron Mitchell and Jennifer Lodge	**Chapel**
Proteomics and Metabolomics	Peter Solomon and Kim Hammond-Kosack	**Kiln**
Stress Signalling	Amir Sharon and Jesus Aguirre	**Nautilus**
Hyphal Tip Growth	Neal Gow and Jurgen Wendland	**Heather**

5:30 - 6:15 pm **Perkins/ Metzenberg Lecture:** Prof. Salomon Bartnicki-Garcia **Merrill Hall**

6:30 Banquet **Crocker Hall**

8:30 pm - 12:30 am Closing party featuring "The Amplified DNA Band" **Merrill Hall**
8:30 pm - 12:30 am Quiet alternative **Surf and Sand Living room**

Sunday, March 20

7:30 am - 8:30 am Breakfast **Crocker Hall**

12:00 pm Check-out

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Ad Hoc Workshops

Wednesday
Neurospora Lunch/Policy Committee
12:15 pm – 1:30 pm
Chapel

Magnaporthe Comparative Genomics
12:15 pm – 1:45 pm
Fred Farr Forum

Thursday
JGI Fungal Genomics Program:Tools and Applications
12:15 pm – 1:30 pm
Merrill Hall

Friday
Colletotrichum Workshop
12:30 pm – 1:45 pm
Scripps

Fungal Genome Tools
12:15 pm – 1:30 pm
Chapel

Concurrent Session Schedules

Concurrent Sessions I

Comparative and Functional genomics **Merrill Hall**
Jason Stajich and Dawn-Anne Thompson

Abstracts for this session begin on page 43

3:00 - 3:20
Minou Nowrousian
Laser capture microdissection, RNA-seq, and mutant genome sequencing: How to use next-generation sequencing to characterize developmental genes in filamentous fungi

3:20 - 3:40
Michael E. Donaldson
Identification and potential function of natural antisense transcripts in the fungal plant pathogen *Ustilago maydis*

3:40 - 4:00
Mikael R. Andersen
A method for accurate prediction of the size of secondary metabolite clusters in *Aspergillus nidulans*

4:00 - 4:20
Sushmita Roy
Arboretum: Thinking about trees to cluster expression across species

4:20 - 4:40 Break

4:40 - 5:00
Jürgen Wendland
The genome sequence for *Eremothecium cymbalariae* establishes a link between the *S. cerevisiae* ancestor and the streamlined genome of *Ashbya gossypii*

5:00 - 5:20
Toni Gabaldón
Fungal genomes as seen through the lens of evolution

5:20 - 5:40
A. Diego Martinez
Comparative Analysis of Dermatophyte Genomes

5:40 - 6:00
Sophien Kamoun
Genome evolution in the Irish potato famine pathogen lineage

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Photobiology **Chapel**

Alexander Idnurm and Luis Corrochano

Abstracts for this session begin on page 46

3:00 - 3:20
Alfredo Herrera-Estrella
Molecular basis of photoconidiation in *Trichoderma atroviride*

3:20 - 3:40
Hun Kim
Regulation of stomatal tropism and infection by light in *Cercospora zeae-maydis*

3:40 - 4:00
Santiago Torres-Martinez
Characterization of *Mucor circinelloides* light-response mutants by high-throughput sequencing

4:00 - 4:20
María Olmedo
Expanding the molecular clock network of *Neurospora crassa*

4:20 - 4:40 Break

4:40 - 5:00
Michael Brunner
Light Sensitivity of First and Second Tier Clock-Controlled Genes in Neurospora.

5:00 - 5:20
Chandrashekara Mallappa
Roles for CSP-1 in Light and Circadian Clock-Regulated Gene Expression

5:20 - 5:40
Maren Hedtke
Light-dependent gene induction in *A. nidulans* requires release of the repressor LreA and binding of the activator FphA

5:40 - 6:00
Gerhard Braus
Light Control of Fungal Development and Secondary Metabolism in *Aspergillus nidulans,*

Fungal Effectors **Heather**
Mark Glijzen and Bart Thomma

Abstracts for this session begin on page 49

3:00 - 3:20
Bart Thomma
Fungal LysM effectors perturb chitin-triggered host immunity

3:20 - 3:40
Jim Beynon
Pathogen effectors reveal a complex host immune network

3:40 - 4:00
Barbara Valent
***Magnaporthe oryzae* effector dynamics during invasion of living rice cells**

4:00 - 4:20
Thorsten Nürnberger
The superfamily of necrosis and ethylene-inducing peptide 1 (Nep1) like proteins (NLPs) harbors cytotoxic and non-cytotoxic, virulence-promoting members

4:20 - 4:40 Break

4:40 - 5:00
Isabelle Fudal
Crystal Structure Of The Avirulence Gene *AvrLm4-7* Of *Leptosphaeria maculans* Illuminates Its Evolutionary And Functional Characteristics. (abstract 187)

5:00 - 5:20
Stephan Wawra
The oomycete RxLR-effectors AVR3a and SpHtp1 show cell type specific import and their RxLR-leaders mediate dimerisation.

5:20 - 5:40
Jan Schirawski
Symptom formation of *Sporisorium reilianum* on maize is mediated by secreted effectors.

5:40 - 6:00
Liliana Cano
Genome analysis of a strain from the UK blue 13 clonal lineage of *Phytophthora infestans* reveals significant genetic and expression polymorphisms in effector genes.

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Symbiosis **Fred Farr Forum**

Alga Zuccaro and Sebastien Duplessis

Abstracts for this session begin on page 52

3:00 - 3:20
Uta Paszkowski
The art and design of harmony: novel arbuscular mycorrhizal factors from cereals

3:20 - 3:40
Uwe Nehls
Aquaporin function in ectomycorrhizal symbiosis: what can we learn from *Laccaria bicolor*?

3:40 - 4:00
Cristina Albarran
Identification of secreted *Glomus intraradices* signals activating the plant symbiotic program

4:00 - 4:20
Pietro Spanu
Genome expansion and gene loss in powdery mildew fungi reveal functional tradeoffs in extreme parasitism

4:20 - 4:40 Break

4:40 - 5:00
Alga Zuccaro
Genome and transcriptome analyses of *Piriformospora indica* provide hints into endophytic life strategies

5:00 - 5:20
Barry Scott
Identification of a transcription regulator controlling in planta hyphal growth of *Epichloë festucae*, a mutualistic symbiont of perennial ryegrass

5:20 - 5:40
Richard Johnson
Metabolomics meets Genomics: Solving the puzzle of how multiple cyclic oligopeptides are synthesised by *epichloae* endophytes via a single ribosomally encoded gene, gigA.

5:40 - 6:00
Carolyn Young
Deconvoluting the *Neotyphodium coenophialum* genome

Evolution of Sex in Fungi **Kiln**
Paul Dyer and Robert Debuchy

Abstracts for this session begin on page 55

3:00 - 3:20
Joe Heitman
Sex in basal fungi: *Phycomyces, Mucor,* and *Rhizopus*

3:20 - 3:40
Tom Martin
Tracing the origin of the fungal alpha1 domain places its ancestor in the HMG-box superfamily

3:40 - 4:00
Jan van Kan.
Unusual features of the *Botrytis cinerea* mating system.

4:00 - 4:20
Sijmen Schoustra
Fitness associated sexual reproduction in *Aspergillus nidulans*

4:20 - 4:40 Break

4:40 - 5:00
Ines Teichert
Fungal developmental networks: Control of fruiting body formation in *Sordaria macrospora.*

5:00 - 5:20
Zheng Wang
Sex-specific expression during asexual development of *Neurospora crassa* under constant light.

5:20 - 5:40
Racquel Sherwood
Regulation of the Meiotic Program in *Candida lusitaniae*.

5:40 - 6:00
Han A. B. Wösten
Regulation of mushroom development

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Evolution of centromeres and centromere function in fungi **Nautilus**
Judith Berman and Kaustuv Sanyal

Abstracts for this session begin on page 58

3:00 - 3:20
Kaustuv Sanyal
Tracing the path of centromere evolution in yeasts

3:20 - 3:40
Michael Frietag
Centromeres in filamentous fungi

3:40 - 4:00
Pallavi Phatale
Genetic analyses of centromere-specific histone H3 proteins from three ascomycetes in *Neurospora crassa*.

4:00 - 4:20
Ajit Joglekar
Outside looking in – A view of the centromere architecture from the kinetochore

4:20 - 4:40 Break

4:40 - 5:00
Xiangwei He
Plasticity and Epigenetic Inheritance of CENP-A Nucleosome Positioning in the Fission Yeast Centromere

5:00 - 5:20
Judith Berman
The requirement for the Dam1 complex is dependent upon the number of kinetochore proteins and microtubules

5:20 - 5:40
Meleah Hickman
Genome-wide identification of replication origins in *Candida albicans*

5:40 - 6:00
Maitreya Dunham
Comparative functional genomics of two *Saccharomyces* yeasts.

Concurrent Sessions II

Fungus-host signaling **Merrill Hall**
Barbara Howlett and Gunther Döhlemann

Abstracts for this session begin on page 61

3:00 - 3:20
Antonio di Pietro
Crosstalk between nutrient and MAPK signalling in the trans-kingdom pathogen *Fusarium oxysporum*

3:20 - 3:40
Carla Eaton
Decoding symbiosis - molecular insights into the basis of grass-fungal interactions

3:40 - 4:00
Tim Friesen
Interaction between dothideomycete toxins and receptors in wheat

4:00 - 4:20
Gunther Doehlemann
Manipulation of plant defense signaling by *Ustilago maydis* effectors

4:20 - 4:40 Break

4:40 - 5:00
Annegret Kohler
The impact of the mycorrhizal symbiosis on the transcriptome of *Laccaria bicolor* and Poplar

5:00 - 5:20
Andreas Thywissen
***Aspergillus fumigatus* conidia modulate the endocytic pathway of alveolar macrophages**

5:20 - 5:40
Christian Voigt
Pathogen-caused release of linolenic acid suppresses plant defense by inhibition of callose synthesis in wheat.

5:40 - 6:00
Thomas Guillemette
Cellular pathways activated in the necrotrophic fungus Alternaria brassicicola in response to camalexin exposure

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Interactions between Fungi and Prokaryotes **Kiln**
Teresa E. Pawlowska & Paola Bonfante

Abstracts for this session begin on page 64

3:00 - 3:20
Diana K. Morales
Understanding chemical crosstalk between bacteria and fungi in biofilms

3:20 - 3:40
Hans-Wilhelm Nützmann
Interaction between *Streptomycetes* and *Aspergillus nidulans*

3:40 - 4:00
Danielle Troppens
Unraveling the biological activities of a bacterial metabolite using *Saccharomyces cerevisiae* and *Neurospora crassa* as model organisms

4:00 - 4:20
Jonathan L. Klassen
Ants, agriculture, and antibiotics

4:20 - 4:40 Break

4:40 - 5:00
Martin Grube
Lichen symbioses as microecosystems

5:00 - 5:20
Aurélie Deveau
Ectomycorrhizal fungi and their bacterial associates: what's new about the mechanisms of their interactions?

5:20 - 5:40
Paola Bonfante
The genome of the obligate endobacterium of an AM fungus reveals an interphylum network of nutritional interactions

5:40 - 6:00
Stephen J Mondo
400 million year old facultative dependence of arbuscular mycorrhizal fungi on Glomeribacter endobacteria.

Emergent fungal diseases **Chapel**
Matt Fisher and John Taylor

Abstracts for this session begin on page 67

3:00 - 3:20
Sarah Gurr
Policy to prevent the transport of pathogens

3:20 - 3:40
Todd Ward
Global molecular surveillance provides a framework for understanding diversity within the *Fusarium graminearum* species complex

3:40 - 4:00
Jean Ristaino
Inferring evolutionary relationships of Phytophthora species in the Ic clade using nuclear and mitochondrial genes

4:00 - 4:20
Rhys Farrer
Comparative genomics of Batrachochytrium dendrobatidis reveals recombination in a single globalised hypervirulent lineage

4:20 - 4:40 Break

4:40 - 5:00
David Blehert
The emergence of *Geomcyes destructans* and bat white-nose syndrome in North America

5:00 - 5:20
Christopher Desjardins
Comparative genomics of human fungal pathogens causing paracoccidioidomycosis

5:20 - 5:40
WenJun Li
Molecular epidemiology of *Cryptococcus gattii* in the Pacific Northwest

5:40 - 6:00
Pierre Gladieux
"Magic traits" drive the emergence of pathogens

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Regulation of Septation During Growth and Development **Heather**
Oded Yarden and Stephan Seiler

Abstracts for this session begin on page 70

3:00 - 3:20
Peter Philippsen
Dynamics of septum formation in *Ashbya gossypii*

3:20 - 3:40
Rosa Mouriño-Pérez
Dynamics of actin and actin binding proteins during septum formation in *Neurospora crassa*

3:40 - 4:00
David Caballero-Lima
Regulation of Septins assembly by Rts1 during *Candida albicans* morphogenesis

4:00 - 4:20
Michael Bölker
Genetic regulation of septation in the dimorphic basidiomycete *Ustilago maydis*

4:20 - 4:40 Break

4:40 - 5:00
Robert-Jan Bleichrodt
Mechanisms of septal closure in filamentous fungi as a response to mechanical damage and stress

5:00 - 5:20
Sandra Bloemendal
The vacuolar membrane protein PRO22 from *Sordaria macrospora* is involved in septum formation in early sexual structures

5:20 - 5:40
Bo Liu
Antagonistic interaction between the RSC chromatin-remodeling complex and the septation initiation network in the filamentous fungus *Aspergillus nidulans*

5:40 - 6:00
Steven D. Harris
Developmental regulation of septum formation in *Aspergillus nidulans*

Secretion, endocytosis & trafficking **Fred Farr Forum**
Brian D. Shaw & Miguel Peñalva

Abstracts for this session begin on page 73

3:00 - 3:20
Meritxell Riquelme
Exocytosis and tip growth in *Neurospora crassa*

3:20 - 3:40
Norio Takeshita
Functional analysis of SPFH domain-containing proteins, Flotillin and Stomatin, in *Aspergillus nidulans*

3:40 - 4:00
Gui Shen
Cryptococcal WASp homolog Wsp1 functions as an effector of Cdc42 and Rac1 to regulate intracellular trafficking and actin cytoskeleton

4:00 - 4:20
Brian Shaw
Imaging actin dynamics in *Aspergillus nidulans* using Lifeact

4:20 - 4:40 Break

4:40 - 5:00
Gero Steinberg
Motor cooperation in membrane trafficking

5:00 - 5:20
Xin Xang
Roles of the dynactin complex in early endosome transport

5:20 - 5:40
Andreas Mayer
Vacuole homeostasis by a balance of membrane fission and fusion

5:40 - 6:00
Miguel Peñalva
The interface between the Golgi and the endosomal system in *Aspergillus nidulans*

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ChIP-chip/ChIP-seq: Protein interactions with DNA **Nautilus**
Thomas Mitchell & Michael Freitag

Abstracts for this session begin on page 76

3:00 - 3:20
Marc Facciotti
Integration of ChIP-Chip/Seq data in a systems biology framework

3:20 - 3:40
Agnieszka Gacek
Histone H3 demethylases are involved in regulating primary and secondary metabolism

3:40 - 4:00
Sinem Beyhan
Regulatory networks that control morphology and virulence in *Histoplasma capsulatum*

4:00 - 4:20
Philippe Lefrancois
Identification of kinetochore-like regions using ChIP-seq and chromosome segregation analyses

4:20 - 4:40 Break

4:40 - 5:00
Abby Leeder
Regulatory networks during cell-cell communication and germling fusion in *Neurospora crassa*

5:00 - 5:20
Rigzin Dekhang
Characterization of circadian clock output pathways regulated by *adv-1* in *Neurospora crassa* using ChIP-seq

5:20 - 5:40
Gopal Subramaniam
ChIP sequencing reveal dual role for the transcription regulator Tri6 in the phytopathogen *Fusarium graminearum*

5:40 - 6:00
James Galagan
Using Chip-Seq to Disect Microbial Regulatory Networks

Education and Public Outreach **Scripps**
Pat Pukkila and Mimi Zolan

Abstracts were not requested for this session.

3:00 - 3:20
Beth Ruedi
The Genetics Society of America and Education: A new initiative, a new program director, and how you can get involved

3:20 - 3:40
Porter Ridley
NSF Broader Impacts from a Program Officer's perspective

3:40 - 4:00
Roundtable discussion of Innovations in Public Outreach and Education led by Pat Pukkila

4:00 - 4:20
Roundtable discussion continued

4:20 - 4:40 Break

4:40 - 5:00
Claire Burns
Making the transition from researcher to teacher: one post-doc's perspective

5:00 - 5:20
Julio Soto
Modernizing a freshman biology sequence with support from HHMI: kinesthetic and inquiry approaches

5:20 - 5:40
Marilee Ramesh
Applying Writing Pedagogy to College Biology Laboratory Assignments

5:40 - 6:00
Andrea Gargas
COMGEN : Fungal genetic analysis as a pedagogical tool

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Concurrent Sessions III

Cell cycle, development and morphogenesis **Fred Farr Forum**
Nick Talbot & Meritxell Riquelme

Abstracts for this session begin on page 80

3:00 - 3:20
Michelle Momany
RNA is asymmetrically localized in *Aspergillus fumigatus*

3:20 - 3:40
Jun-ya Shoji
Macroautophagy-mediated degradation of whole nuclei in the filamentous fungus *Aspergillus oryzae*

3:40 - 4:00
Hye-Seon Kim
Comparative analysis of hyphal Ca^{2+} dynamics in three *Fusarium* species and the role of calcium channel genes in the generation of hyphal tip Ca^{2+} pulses

4:00 - 4:20
Nick Talbot
Investigating the role of the septin gene family in *Magnaporthe oryzae* during rice infection

4:20 - 4:40 Break

4:40 - 5:00
Ane Sesma
RNA-binding protein mediates *M. oryzae* cellular differentiation and plant infection through regulation of mTOR, HOG1, cAMP, and pH signalling cascades

5:00 - 5:20
Ulrich Kück
The velvet-like complex from *Penicillium chrysogenum*: A regulatory network of five subunits controls secondary metabolism and morphogenesis

5:20 - 5:40
Jörg Kämper
Regulatory networks coordinating nuclear division and pathogenic development in *Ustilago maydis*

5:40 - 6:00
Nandini Shukla
Identification of a microtubule associating protein that interacts with nuclear pore complex proteins during mitosis

Host Selective Toxins **Nautilus**
B. Gillian Turgeon & Tim Freisen

Abstracts for this session begin on page 83

3:00 - 3:20
Gillian Turgeon
Comparative genomics of host selective toxin producing pathogens of cereals

3:20 - 3:40
Tom Wolpert
A 'Born Again' fungal virulence effector

3:40 - 4:00
Motochiro Kodama
Pathogenicity chromosomes in host-specific toxin-producing *Alternaria* species

4:00 - 4:20
Wanessa Wight
Histone deacetylase inhibitor HC-toxin from *Alternaria jesenskae*

4:20 - 4:40 Break

4:40 - 5:00
Lynda Cuiffetti
Same fungus, two different host-selective toxins: perceptions and outcomes

5:00 - 5:20
Zhaohui Liu
A novel, cysteine-rich fungal effector triggers light-dependent susceptibility in the wheat-*Stagonospora nodorum* interaction

5:20 - 5:40
Delphine Vincent
A proteomics approach to dissect SnToxA effector mode-of-action in wheat

5:40 - 6:00
Richard Oliver
Quantitative variation in activity of ToxA haplotypes from *Stagonospora nodorum* and *Pyrenophora tritici-repentis* refines the distinction between biotrophic and necrotrophic interactions

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Fungicides & Antifungals **Chapel**
Leah Cowen & Sabine Fillinger

Abstracts for this session begin on page 86

3:00 - 3:20
Hugo Wurtele
Control of the Chromosome Acetylation Cycle as a novel anti-fungal therapeutic strategy

3:20 - 3:40
Scott Erdman
Genome-wide screens using a natural product saponin identify three PDR pathway target genes, PDR19, PDR20 and PDR21, which influence lipid homeostasis and membrane permeability in *Saccharomyces cerevisiae*.

3:40 - 4:00
Popchai Ngamskulrungroj
Characterization of Fluconazole-related Chromosomal duplication in *Cryptococcus neoformans*

4:00 - 4:20
Danièle Debieu
Natural and acquired fenhexamid resistance in *Botrytis* spp. What's the difference?

4:20 - 4:40 Break

4:40 - 5:00
Paul E. Verweij
Resistance to medical triazoles and exposure to azole fungicides in the opportunistic fungus *Aspergillus fumigatus*

5:00 - 5:20
Michaela Leroch
Mechanisms of multiple fungicide resistance in *Botrytis cinerea* populations from vineyards and strawberry fields

5:20 - 5:40
Nalu Peres
Remodeling of the fungal cell wall contributes to Fludioxonil and Ambruticin resistance in the dermatophyte *Trichophyton rubrum*

5:40 - 6:00
Sheena D. Singh-Babak
Global analysis of the evolution and mechanism of echinocandin resistance in a series of *Candida glabrata* clinical isolates

Biobased products, biofuels, and bioenergy **Merrill Hall**
Johnathan Walton & Peter Punt

Abstracts for this session begin on page 89

3:00 - 3:20

N. Louise Glass
Systems biology approaches to understanding plant cell wall degradation in a model filamentous fungus

3:20 - 3:40
Goutami Banerjee
Improving fungal enzymes for biomass conversion

3:40 - 4:00
Antonius J.A. van Maris
Engineering of *Saccharomyces cerevisiae* for efficient alcoholic fermentation of plant biomass hydrolysates

4:00 - 4:20
Dana Wohlbach
Comparative genomics of xylose-fermenting fungi to enhance microbial biofuel production

4:20 - 4:40 Break

4:40 - 5:00
Marcus Hans
Production of pravastatin by metabolically engineered *Penicillium chrysogenum* cells

5:00 - 5:20
Dominik Mojzita
Oxido-reductive metabolism of L-arabinose and D-galactose in filamentous fungi: Metabolic crosstalk versus specific enzymes

5:20 - 5:40
Gary Foster
Biobased antibiotics from basidios: Identification and manipulation of the pleuromutilin gene cluster from *Clitopilus passeckerianus*

5:40 - 6:00
Niels B. Hansen
Production of dicarboxylic acids by *Aspergillus carbonarius*, the engineering of a novel biochemical cell factory

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High Throughput Methods for Filamentous Fungi **Heather**
Marco Berg-van-den & Aric Wiest

Abstracts for this session begin on page 92

3:00 - 3:20
Marco van den Berg
New methods for High Throughput generation of precise gene knock-outs of *Penicillium chrysogenum*

3:20 - 3:40
Suzana Car
Fungal enzymes for biomass deconstruction

3:40 - 4:00
Kevin McCluskey
From one to ten thousand mutants: the development of high-throughput methods at the Fungal Genetics Stock Center

4:00 - 4:20 Richard Wilson
Comparative phenotyping coupled with high throughput forward genetics and gene deletion strategies reveals novel determinants of pathogenicity in the rice blast fungus *Magnaporthe oryzae*

4:20 - 4:40 Break

4:40 - 5:00 Luis Larrondo
A reverse and forward genetic clock-screening strategy to identify new circadian regulators in *Neurospora crassa*

5:00 - 5:20
Suzanne Noble
Screens of a *Candida albicans* homozygous gene disruption library reveal novel regulators of virulence and commensalism

5:20 - 5:40
Masayuki Machida
High throughput analysis of gene function by comparative genomics

5:40 - 6:00
Doris Roth
Secretome discovery reveals lignocellulose degradation capacity of the ectomycorrhizal fungus *Paxillus involutus*

Population Genomics **Kiln**
James Anderson & Eva Stukenbrock

Abstracts for this session begin on page 95

3:00 - 3:20
Sarah M. Schmidt
Evolution of lineage-specific chromosomes in the *Fusarium oxysporum* species complex

3:20 - 3:40
Rodrigo A. Olarte
Sexual recombination and the possibility of cryptic heterokaryosis in *Aspergillus flavus*

3:40 - 4:00
Christopher E. Ellison
Population genomics and local adaptation in *Neurospora crassa* isolates from the Caribbean Basin

4:00 - 4:20
Eva H. Stukenbrock
The making of a new pathogen: Insight from comparative population genomics of the domesticated wheat pathogen *Mycosphaerella grmainicola* and its wild sister species

4:20 - 4:40 Break

4:40 - 5:00
Alexander Idnurm
Sex determination in the original sexual fungus

5:00 - 5:20
Jason E Stajich
Population genomics of the amphibian pathogen *Batrachochytrium dendrobatidis* from genome resequencing

5:20 - 5:40
Anna Selmecki
How polyploidy and aneuploidy impact the speed of adaptation

5:40 - 6:00
Takao Kasuga
Host induced epigenetic alteration in *Phytophthora ramorum*

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Dimorphic Transitions **Scripps**
Julia Koehler & Anne Dranginis

Abstracts for this session begin on page 98

3:00 - 3:20
Yasin F Dagdas
Septin-mediated morphological transitions during plant infection by the rice blast fungus

3:20 - 3:40
Amritha S. Wickramage
***RIG1*, a gene essential for pathogenicity in *Magnaporthe oryzae*, is representative of Gti1_Pac2 family members required for invasive growth in fungal pathogens of plants and animals**

3:40 - 4:00
Haoping Liu
Hyphal development in *Candida albicans* requires two temporally linked regulations of promoter chromatin for initiation and maintenance

4:00 - 4:20
Xiaorong Lin
Characterization of ZNF2 as a master regulator for hyphal morphogenesis and virulence in Cryptococcus neoformans

4:20 - 4:40 Break

4:40 - 5:00
Soo Chan Lee
The calcineurin pathway governs dimorphic transition in the pathogenic zygomycete *Mucor circinelloides*

5:00 - 5:20
Alex Andrianopoulos
Shared regulation during asexual development and dimorphic switching in the human fungal pathogen *Penicillium marneffei*.

5:20 - 5:40
João Menino
Morphological heterogeneity *of Paracoccidioides brasiliensis:* characterization and relevance of the Rho-like GTPase *Pbcdc42*

5:40 - 6:00
Peter N. Lipke
Cell adhesion nanodomains result from amyloid formation on fungal cell surfaces

Concurrent Sessions IV

Genome Defense Mechanisms, Epigenetics and RNAi **Merrill Hall**
Rodolfo Aramayo & Yi Liu

Abstracts for this session begin on page 101

2:00 - 2:20
Eric Selker
Control of DNA methylation in Neurospora

2:20 - 2:40
Rosa M. Ruiz-Vázquez
Functions of *Mucor circinelloides* RNA-dependent RNA polymerases in the Dicer-dependent and Dicer-independent regulation of endogenous mRNAs

2:40 - 3:00
Matt Sachs
Genome-wide analysis of *Neurospora crassa* transcripts regulated by the nonsense-mediated mRNA decay pathway

3:00 - 3:20
Patricia J. Pukkila
Domains of DNA methylation in *Coprinopsis cinerea* (*Coprinus cinereus*)

3:20 - 3:40 Break

3:40 - 4:00
Donald Nuss
Severe symptoms observed for infected RNA silencing mutants of *Cryphonectria parasitica* are associated with a central region of the Hypovirus genome

4:00 - 4:20
Qiuying Yang
Diverse Pathways Generate Aberrant RNAs, MicroRNA-like RNAs and Dicer-Independent Small Interfering RNAs in Fungi

4:20 - 4:40
Patrick Shiu
Mapping and characterization of the Neurospora Spore killer elements

4:40 - 5:00
Rodolfo Aramayo
Meiotic Silencing in Neurospora

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Systems and Synthetic Biology **Fred Farr Forum**
Han de Winde & Merja Penttilä

Abstracts for this session begin on page 104

2:00 - 2:20
Rachel Brem
Pathway evolution in Saccharomycetes

2:20 - 2:40
Jurg Bahler
Genome Regulation in Fission Yeast

2:40 - 3:00
Charles Hall
The development of genetics and genomics for analysis of complex traits in the model filamentous fungus, *Neurospora crassa*

3:00 - 3:20
Tiina Pakula
A genomics based search for regulators for enzyme production

3:20 - 3:40 Break

3:40 - 4:00
Jean-Marc Daran
Systems-based analysis of adipic acid catabolism in *Penicillium chrysogenum*

4:00 - 4:20
Charissa de Becker
Systems analysis of hyphal heterogeneity in *Aspergillus niger*

4:20 - 4:40
Jeremy Zucker
Genome-scale metabolic reconstruction and curation of the filamentous fungus *Neurospora crassa*

4:40 - 5:00
Guido Melzer
Systems-level design of filamentous fungi; integration of in silico flux modes and in vivo pathway fluxes towards desired production properties

Fungi that infect humans **Chapel**
Aaron Mitchell & Jennifer Lodge

Abstracts for this session begin on page 107

2:00 - 2:20
Leah Cowen
Hsp90 Governs Drug Resistance and Dispersion of Fungal Biofilms

2:20 - 2:40
Tamara Doering
A systems approach to regulation of a fungal virulence factor

2:40 - 3:00
Bettina Fries
Allergen 1 and 2 constitute a novel class of virulence associated genes that are regulated by phenotypic switching

3:00 - 3:20
Lorina Baker Boomhower
Chitosan is necessary to establish *Cryptococcus neoformans* infection

3:20 - 3:40 Break

3:40 - 4:00
Brendan Cormack
***Candida glabrata* sub-telomeres and virulence gene evolution**

4:00 - 4:20
Leona Campbell
Analysis of the secretomes of *Cryptococcus gattii* strains with different virulence profiles

4:20 - 4:40
Ashraf Ibrahim
Iron is critical for mucormycosis pathogenesis in the diabetic ketoacidotic host

4:40 - 5:00
Adnane Sellam
Insight into transcriptional regulatory mechanisms controlling filamentation in *Candida albicans* under hypoxia

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Proteomics and Metabolomics **Kiln**
Peter S. Solomon & Kim Hammond-Kosack

Abstracts for this session begin on page 110

2:00 - 2:20
Silas Vilas-Boas
A metabolomic study of *Candida albicans* morphogenesis reveals the potential role of the cell redox balance on the morphological transition

2:20 - 2:40
Martin Urban
Metabolome phenotyping of *Fusarium graminearum* wt and single gene deletion mutant strains affected in virulence under DON-inducing and non-inducing conditions

2:40 - 3:00
Sabine Fillinger
The fludioxonil induced phosphoproteomes of the phytopathogenic fungi *Alternaria brassicicola* and *Botrytis cinerea*

3:00 - 3:20
Peter Punt
A functional genomics study of extracellular protease production by *Aspergillus niger*

3:20 - 3:40 Break

3:40 - 4:00
Stephen Strelkov
A proteomics approach to understanding virulence in *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat

4:00 - 4:20
Dee Carter
The *Cryptococcus gattii* proteome in growth and response to fluconazole

4:20 - 4:40
Liam Cassidy
A quantitative proteomic analysis of the wheat pathogen *Stagonospora nodorum* during sporulation

4:40 - 5:00
William Franck
A Mass Spectrometry Based Examination of the *Magnaporthe oryzae* Proteome During Appressorium Development

Stress signaling, ROS signaling, and programmed cell death **Nautilus**
Jesus Aguirre & Amir Sharon

Abstracts for this session begin on page 113

2:00 - 2:20
Kaz Shiozaki
Signaling mechanisms that sense and combat oxidative stress in *Schizosaccharomyces pombe*.

2:20 - 2:40
Axel A. Brakhage
The CCAAT-binding complex coordinates the oxidative stress response in eukaryotes

2:40 - 3:00
Alexander Lichius
On the role of NOX-derived ROS during cell fusion in *Neurospora crassa*

3:00 - 3:20
Nicole Donofrio
The *HYR1* gene in the rice blast fungus functions to tolerate plant-produced reactive oxygen species during infection

3:20 - 3:40 Break

3:40 - 4:00
Marty Dickman
Tipping the Balance*: Sclerotinia sclerotiorum* regulates autophagy, apoptosis and disease development by manipulating the host redox environment

4:00 - 4:20
Heinz D. Osiewacz
Scavenging of reactive oxygen species (ROS) as part of a hierarchical network of mitochondrial pathways involved in aging and lifespan control

4:20 - 4:40
Amir Sharon
Apoptotic fungal cell death mediates host invasion by pathogenic fungi

4:40 - 5:00
Margaret E. Katz
Evidence that HxkC, an *Aspergillus nidulans* mitochondrial hexokinase-like protein, is anti-apoptotic

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Hyphal tip growth **Heather**
Neil Gow & Jürgen Wendland

Abstracts for this session begin on page 113

2:00 - 2:20
Zhenbiao Yang
Mechanisms of tip growth in pollen tubes

2:20 - 2:40
Reinhard Fischer
Organization and role of the microtubule cytoskeleton in *Aspergillus nidulans*

2:40 - 3:00
Alex Brand
Role of the Cdc42 polarity complex in hyphal tip steering

3:00 - 3:20
Yue Wang
Linking the Hgc1-Cdc28 CDK to the polarity machinery in *Candida albicans* hyphal development

3:20 - 3:40 Break

3:40 - 4:00
Marianna Feretzaki
Role of hyphal development in virulence of human fungal pathogen *Cryptococcus neoformans*

4:00 - 4:20
Daniel Lanver
The signaling mucin Msb2 is processed into cellular and extracellular fragments during its function in appressorium formation of *Ustilago maydis*

4:20 - 4:40
Christine Voisey
Intercalary growth in vegetative hyphae of the ryegrass endophyte *Epichloë festucae*

4:40 - 5:00
Daigo Takemoto
Polarity proteins Bem1 and Cdc24 are components of the filamentous fungal NADPH oxidase complex

Abstracts for Plenary Session Talks

Plenary Session I: Genome Evolution
Chair: Ralph Dean, North Carolina State University

Fruiting body development and transcriptomics in Neurospora species
Nina Lehr, Zheng Wang, Francesc Lopez-Giraldez, Marta Farre, Frances Trail, and Jeffrey P. Townsend

Shifts in gene expression drive differentiation of tissues and the evolution of new morphologies in multicellular organisms. However, studies linking the evolution of gene expression and the evolution of development are difficult in complex organisms whose gene expression depends on environmental as well as genetic differences. We have carefully controlled the environment and developed novel techniques to examine microscopic phenotype and to assay genome-wide gene expression during perithecial development using next-generation sequencing in three species of Neurospora: the heterothallics N. crassa and N. discreta, and the pseuodohomothallic N. tetrasperma. We have revealed elements of the underlying transcriptional program of fruiting body development. These developmental processes are fundamental to sexual reproduction, recombination, and to the adaptive dynamics of pathogens and hosts. This information, by comparison to other species such as Fusarium, will be used to estimate the ancestral evolutionary transitions that resulted in the shifts in morphology and ecology.

Diversity and evolution of the wood-decay apparatus in saprotrophic Agaricomycotina. David Hibbett[1], Dan Cullen[2], Francis Martin[3], Daniel C. Eastwood[4], Antonio Pisabarro[5], Igor Grigoriev[6]. [1]Biology Department, Clark University, Worcester, Massachusetts 01610 USA. [2]USDA Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI 53726 USA. [3]UMR INRA/UHP 1136, Interactions Arbres/Micro-Organismes, INRA-Nancy, 54280 Champenoux, France. [4]College of Science, University of Swansea, Singleton Park, Swansea SA2 8PP Wales UK. [5]Department of Agrarian Production, Public University of Navarre, 31006 Pamplona, Spain. [6]US DOE Joint Genome Institute, Walnut Creek, California, USA.

The Agaricomycotina is a clade of over 21000 described species that includes mushrooms, jelly fungi, and certain yeasts. Most Agaricomycotina are either saprotrophs (decayers) or ectomycorrhizal (ECM) symbionts, but the group also contains lichen-formers, insect symbionts, plant and animal pathogens, and mycoparasites. Saprotrophic Agaricomycotina are of great interest for biotechnological applications, as they are active and abundant degraders of all classes of plant tissues, including the recalcitrant lignin fraction. The Saprotrophic Agaricomycotina Project (SAP) seeks to generate whole genome sequences of thirty species, emphasizing white and brown rot wood decay taxa, with a focus on families of genes encoding decay-related carbohydrate-active enzymes (CAZYs) and oxidoreductases. Analyses of the first nine SAP genomes along with exemplars of other groups of Fungi suggest that white rot species are enriched in decay-related CAZYs and oxidoreductases relative to brown rot and ECM species. Gene tree/species tree reconciliation analyses suggest that fungal class II peroxidases, which function in lignin degradation, have expanded independently in multiple white rot lineages. Preliminary molecular clock analyses suggest that initial duplications of fungal class II peroxidases occurred early in the evolution of Agaricomycetes, which is consistent with the fossil record of white rot wood decay in the Triassic period.

The birth, evolution and death of metabolic pathways in fungi
Antonis Rokas, Department of Biological Sciences, Vanderbilt University, VU Station B #35-1634, Nashville, TN 37235

Fungal species are typically saprobes, embedded in their food sources and required to digest their food externally in the presence of competitors. To survive in such a hostile environment, fungi have evolved a bewildering diversity of metabolic capabilities. Importantly, this phenotypic diversity is reflected in their genomes. Thus, by examining the fungal DNA record we can gain valuable insights into the evolution of their metabolic lifestyles. Using a variety of evolutionary and functional genomic techniques, we have begun a systematic investigation of metabolic pathways across 100 fungal genomes that span the entire fungal kingdom, which are beginning to elucidate their origins and evolutionary fate. For example, a notable characteristic of fungal metabolic pathways is that their genes are often physically clustered. We have discovered that such clustered fungal metabolic pathways can evolve independently, that they are easier lost than their non-clustered counterparts, but also that they are also more likely to transfer horizontally between species. Surprisingly, such transfers can move even the largest known metabolic pathways. For example, we found that a complete sterigmatocystin gene cluster in *Podospora anserina*, which contains 24 genes and spans 57 Kb, was horizontally transferred from *Aspergillus*. We conclude that the fungal DNA record is a treasure-trove for understanding adaptation to changing environments through the acquisition and loss of metabolic capacities.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Evolution of hypoxic regulation in *Candida* species

School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.

Exposure of fungi to hypoxic (low oxygen) conditions results in numerous transcriptional changes, including an increase in expression of genes required for ergosterol synthesis, metal transport, and glycolysis, and reduction in expression of respiratory genes. Within the Pezizomycotina (filamentous ascomycetes), expression of ergosterol genes is regulated by members of the bHLH SREBP (Sterol Regulatory Element Binding proteins) family. The function of SREBP proteins in regulating sterol synthesis is generally conserved from mammals to fungi, though with some notable variations. For example, the sterol-sensing protein SCAP protein has been lost from the Eurotiomycetes (including *Aspergillus* species). There are no SREBP family members in most members of the Saccharomycotina (Candida and Saccharomyces species). In these lineages, SREBP is apparently functionally replaced by Upc2, a zinc-finger containing transcription factor. We show that the function of Upc2 is conserved in members of the pathogenic *Candida* clade (*C. albicans* and *C. parapsilosis*). Upc2 regulates expression of sterol synthesis genes in low oxygen conditions. In several species that have undergone whole genome duplication (including *S. cerevisiae* and *C. glabrata*) there are two paralogs of Upc2, called Upc2 and Ecm22. In *S. cerevisiae*, Upc2 plays a major role in hypoxic regulation, and Ecm22 has a minor role. In *C. glabrata* however, Ecm22 has the major role, and the most likely paralog of Upc2 does not regulate expression in hypoxia. *S. cerevisiae* cells also sense oxygen via levels of heme. Biosynthesis of heme regulates the activity of the transcription factor Hap1, which controls expression of the repressor Rox1. When oxygen levels drop, Rox1 is not expressed and therefore no longer represses expression of >100 hypoxic genes. Rox1 in an HMG–domain protein that is apparently restricted to the Saccharomycotina. We show that Rox1 is present in many of the Saccharomycotina species, together with a Rox1-like protein, Rxl1. There is no Rxl1 protein in the *S. cerevisiae* genome, and Rox1 plays a major role in repression of hypoxic genes. *C. glabrata* in contrast has lost Rox1, but maintained Rxl1. Surprisingly the *C. glabrata* Rxl1 gene is also involved in hypoxic regulation. In *C. albicans*, the Rxl1 paralog has no role in hypoxic regulation (and Rox1 is lost). We are currently investigating the role of Rox1 and Rxl1 in species where both genes are present.

Genome dynamics in the *Fusarium oxysporum* species complex. Martijn Rep[1], Li-Jun Ma[3], H. Corby Kistler[3], Charlotte van der Does[1,4], Ido Vlaardingerbroek[1], Shermineh Shahi[1], Petra Houterman[1], Ben Cornelissen[1] and Sarah M. Schmidt[1]. [1] Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, P.O. Box 94215, 1090 GE Amsterdam, The Netherlands. [2] Broad Institute, Cambridge, MA /University of Massachusetts Amherst, MA, USA. [3] Plant Pathology, College of Food, Agricultural and Natural Resource Sciences, Minneapolis, USA. [4] Current affiliation: Department of Plant Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany.

The *Fusarium oxysporum* species complex is a collection of apparently asexually propagating clonal lines. The complex is well known for its genetic diversity, ecological versatility including host-specific pathogenicity, karyotope variability and transposon richness. It is now clear that these phenomena are correlated to the presence of *lineage-specific* (LS) genomic regions of many megabases as an addition to a conserved 'core' genome. These regions are present as either entire LS chromosomes or extensions to core chromosomes and can constitute as much as 25% of the entire genome. The LS genomic regions contain the bulk of the transposons, are extremely variable in organisation even within a clonal line and highly diverse in gene composition between clonal lines. Some LS chromosomes contain genes involved in virulence towards plants and can be transferred horizontally between clonal lines. Through comparative genomics of strains from different clonal lines and with different host-specificity we aim to better understand the evolutionary dynamics of the LS metagenome of the *Fusarium oxysporum* species complex.

Plenary Session II: Symbioses
Chair: Francis Martin, INRA, France

The effectors of smut fungi. Regine Kahmann, Max Planck Institute for Terrestrial Microbiology, D-35043 Marburg, Germany. kahmann@mpi-marburg.mpg.de

Smut fungi like *Ustilago maydis* are biotrophic pathogens that need living plant tissue to complete their life cycle. To establish a compatible interaction smut fungi secrete a large arsenal of protein effector molecules that either function in the apoplast or are translocated to plant cells. Many of the respective effector genes reside in gene clusters that are highly divergent in related pathogens parasitizing on either the same or on different hosts. This suggests that effectors from this group have diverged in response to rapidly evolving host targets to avoid recognition. Other effectors are highly conserved, suggesting that they interact with conserved targets in different host plants. Besides this comparative genomics approach I will focus on the functional analysis of two effectors that both suppress plant defense responses but do so by different mechanisms. For Stp1 a mutational analysis will be presented that reveals distinct functional domains in the N- and C-terminal parts of the molecule. For Cmu1, a secreted chorismate mutase, I will provide evidence that this enzyme is taken up by plant cells and primes the host metabolism prior to colonization by *U. maydis.*

Root sweet home: signaling in the arbuscular mycorrhizal symbiosis. Natalia Requena, Silke Kloppholz and Hannah Kuhn. Plant-Microbe Interactions Dept., Karlsruhe Institute of Technology (KIT) natalia.requena@kit.edu

Biotrophic fungi interacting with plants establish long-term relationship with their hosts to fulfil their life cycles. In contrast to necrotrophs, biotrophic fungi need to sort out the defense mechanisms of the plant to develop within the host and feed on living cells. Arbuscular mycorrhizal (AM) fungi are obligate biotrophs of plant roots. They establish stable associations that can last the life span of the plant providing this with mineral nutrients in exchange for photoassimilates. This is only possible through a complex exchange of molecular information between both partners that allows the life in symbiosis. In the meantime it is generally accepted that microbial pathogens produce and deliver a myriad of effector proteins to hijack the cellular program of their hosts. We investigated whether in the mutualistic arbuscular mycorrhizal symbiosis the delivery of fungal effector molecules play a role in short cutting the defense program of the host. We show here that the fungus *G. intraradices* secretes a protein, SP7, that interacts with an ERF pathogenesis-related transcription factor in the plant nucleus. The expression of this ERF transcription factor is highly induced in roots by infection with the fungal pathogen *Colletotrichum trifolii* but only transiently during early mycorrhiza colonization. SP7 constitutively expressed *in planta* leads to higher mycorrhization while reduces the levels of *C. trifolii*-mediated ERF induction. Furthermore, expression of SP7 in the rice blast fungus *M. oryzae* attenuates root decay symptoms. Taken together these results suggest that SP7 is an effector that contributes to establish/maintain the biotrophic status of arbuscular mycorrhizal fungi in roots by counteracting the early plant immune response.

Artificial selection of *Termitomyces* fungi by termites. Duur K. Aanen. Laboratory of Genetics, Wageningen University, Wageningen, The Netherlands

In termites, a single transition has occurred to the cultivation of edible mushrooms, in the subfamily Macrotermitinae, whose ancestor domesticated basidiomycete fungi (genus *Termitomycees*) ca 30 mya. The fungi produce most of the food for the termites, while the termites provide optimal stable growth conditions for the fungi. Despite the obligate nature of this relationship, both partners still reproduce and disperse independently and potentially can form associations with many alternative genotypes.
In my talk, I will discuss recent findings on the evolution of this mutualism, focusing on how termites can select fungi during several stages of their life cycle. First, studies on large-scale co-evolutionary patterns have shown that interaction specificity occurs, mainly at the genus level. I will discuss how specificity may arise, and how partners select each other. Second, experimental work shows how colonies succeed in propagating only a single heterokaryon of their *Termitomyces* symbiont, despite initiating cultures from genetically variable sexual spores from the habitat at the start of a colony. This exclusive lifetime association of a host colony with a single fungal symbiont hinders the evolution of cheating. However, I will argue that continuous `artificial' selection of the fungus by termites during a colony's life time is required in order to keep the fungus productive.

DOI: 10.4148/1941-4765.1066

Microbial Interactions in a Phytopathogenic Bacterial-Fungal Symbiosis. Christian Hertweck. Leibniz Institute for Natural Product Research and Infection Biology (HKI), and Friedrich Schiller University, Jena, Germany

Pathogenic fungi generally exert their destructive effects through virulence factors. An important example is the macrocyclic polyketide rhizoxin, the causative agent of rice seedling blight, from the fungus *Rhizopus microsporus*. The phytotoxin efficiently binds to rice ß-tubulin, which results in inhibition of mitosis and cell cycle arrest.
By a series of experiments we could unequivocally demonstrate that rhizoxin is not biosynthesized by the fungus itself, but by endosymbiotic bacteria of the genus *Burkholderia*. Our unexpected findings unveil a remarkably complex symbiotic-pathogenic alliance that extends the fungus–plant interaction to a third, bacterial key player. In addition, we were able to culture the symbionts to produce antitumoral rhizoxin derivatives, and to elucidate the biosynthesis of the toxin. A second example for the formation of a 'mycotoxin' by endofungal bacteria is the cyclopeptide rhizonin.
Surprisingly, in the absence of bacterial endosymbionts the fungal host is not capable of vegetative reproduction. Formation of sporangia and spores is only restored upon re-introduction of endobacteria. The fungus has become totally dependent on endofungal bacteria, which in return provide a highly potent toxin for defending the habitat and accessing nutrients from decaying plants.
This talk highlights the significance of toxin-producing endofungal bacteria in the areas of ecology, medicine, and nutrition. Furthermore, progress in studying the molecular basis for the development and persistence of this rare microbial interaction is presented.

Evolutionary origins of endophytic fungi. A. Elizabeth Arnold, School of Plant Sciences, The University of Arizona, Tucson, AZ 85721

Endophytic fungi occur in all lineages of land plants, and in lichens as 'endolichenic fungi,' in biomes ranging from tropical forests to dry deserts and Arctic tundra. Despite their ubiquity little is known about the ecological roles and evolutionary origins of these highly diverse, avirulent symbionts. Synthesizing culture-based and culture-free studies from >150 species of plants and lichens in 10 biogeographic provinces, *in vitro* and *in vivo* assessments of functional traits, and ancestral state reconstructions derived from robust phylogenies, I will address three main questions: (1) What are the evolutionary origins of these ubiquitous symbionts? (2) To what degree do they demonstrate co-evolution with their hosts? (3) How do these previously unknown fungi clarify the structure of the fungal tree of life, and inform our understanding of major trophic and ecological transitions in the most species-rich lineages of Fungi?

Plenary Session III: Growth and Reproduction
Chair: Steve Harris, University of Nebraska

The developmental determinacy and reprogramming of *Sclerotinia sclerotiorum* apothecia. Jeffrey Rollins, Department of Plant Pathology, University of Florida, Gainesville, FL 32611

The Ascomycota produce a great diversity of sexual fruiting body forms united by the requirement for cooperation between maternally-derived haploid hyphae and dikaryotic hyphae. The fruiting body itself is a determinate structure; likewise, within the context in which they are produced, participating tissues take on specialized determinate functions. My lab is using the homothallic Leotiomycetes fungus *Sclerotinia sclerotiorum* to explore the underlying genetic regulators and biochemical signals that condition tissue determinacy and allow for the spatial and temporal coordination of proper apothecium development. We have taken candidate gene approaches guided by microarray data in addition to forward genetic screens with T-DNA-tagged lines to identify candidate factors that perceive and integrate external signals as well as the endogenous regulators affecting apothecial development. Evolutionarily conserved as well as Eumycota-specific factors are beginning to be revealed that function in pattern formation and tissue determinacy. Emerging models based on this work are allowing us to test new hypothesis that will refine our understanding of the cooperative and robust development of the apothecium.

Nuclear anarchy in multinucleate *Ashbya gossypii* cells. Cori D'Ausilio and Amy S. Gladfelter. Department of Biological Sciences, Dartmouth College, Hanover, NH

Genetically identical cells that share the same environment commonly vary in the length of their cell division cycles. In some multinucleate cells, variable and asynchronous nuclear division cycles are even observed between nuclei residing in the same cytosol. How variation is generated and tolerated within the tight regulatory controls of the cell cycle is not well understood. We study nuclear asynchrony in a model filamentous hemi-ascomycete, *Ashbya gossypii*. Nuclear asynchrony arises even in the earliest divisions of *Ashbya* germlings indicating that timing variability is an intrinsic feature of the cell cycle . Additionally, we see that sisters born of the same mitosis diverge in their cell cycle times early in G1. To determine if nuclear timing differences between sisters arises due to a stochastic or systematic process, we performed large scale pedigrees to evaluate timing of many related nuclei. We use a specialized statistical test to determine the nature of associations between the division times of related nuclei. Remarkably, we find a systematic and positive association between sister nuclei division cycle timing indicating that while sisters differ in absolute cycle times, they are more similar to each other than other nuclei. To determine if this relationship was due to common cytoplasmic or nuclear signals shared by sisters we determined whether sister nuclei share the same cytoplasm after birth. Tracking the trajectories of sister pairs demonstrates that even sisters that travel far apart (>50 microns) retain similar cycle times. This indicates that a nuclear intrinsic feature is inherited and leads to a timing connection between genetically related but physically distant nuclei. We have begun a search for nuclear intrinsic features that unite sister nuclei in time and are investigating how nuclear size and genome instability contribute to asynchronous division in multinucleate cells.

Connections between cell cycle, morphogenesis and pathogenicity in fungi: the case of *Ustilago maydis*. José Pérez-Martín. Centro Nacional de Biotecnología-CSIC, Madrid, Spain

Ustilago maydis, a plant pathogen, is worthy of attention since it is perfectly suited to analyze the relationships between cell cycle, morphogenesis and pathogenicity. Previous to infect the plant, *U. maydis* suffers a strong yeast-to-hypha transition that is triggered by the mating of sexually compatible haploid cells. After cell fusion, a straight hypha called infective tube is formed, which is composed of a single dikaryotic cell that is cell cycle arrested, and that supports a strong polarized growth. The arrest is transient and eventually the filament manages to enter the plant tissue, where it starts to proliferate as a dikaryotic filament. Therefore the induction of the pathogenic program implies not only strong morphological changes (bud to hypha transition) but also genetic changes (haploid to dikaryotic transition). Subsequently, an accurate control of the cell cycle and morphogenesis is predicted during these transitions. Our laboratory have been focused in the study of the connections between cell cycle regulation and the induction of pathogenic program that correlates with the induction of the infective tube. In this communication, we will summarize our current knowledge of cell cycle regulation in *U. maydis* and how this regulation is related to the different morphogenetic changes produced during the pathogenic program. We also would like to discuss how manipulation of fungal cell cycle could produce strong morphological effects and hypothesize how these could be in principle be applied to modify the morphology of filamentous fungi.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Dissecting COT1 NDR kinase regulation and signaling in *Neurospora crassa*. Sabine März[1], Anne Dettmann[1], Carmit Ziv[2], Oded Yarden[2], and Stephan Seiler[1]. [1] Institute for Microbiology and und Genetics, University of Göttingen, Germany. [2] Department of Plant Pathology and Microbiology, Hebrew University of Jerusalem, Israel

NDR kinases are important for cell polarity and differentiation in various organisms, yet the regulation of their activity and their integration into a cellular signaling context is still elusive. The NDR kinase COT1 is essential for hyphal elongation and inhibits excessive branch formation in *Neurospora crassa*, while related kinases are important for fungal pathogenicity and differentiation in other fungi. We demonstrate that COT1 is regulated through differential interaction with several scaffolding proteins and coactivators and show that Ser419 and Thr589 are key regulatory phosphorylation sites that determine COT1's activity *in vitro* and its function and membrane association *in vivo*. Specifically, we determined that the NDR kinase COT1 forms homodimers. These kinase dimers interact through a N-terminal extension, which is also necessary for the interaction of COT1 with MOB2A and MOB2B, suggesting the presence of mutually exclusive homo- and heterocomplexes. Initial activation of the kinase requires autophosphorylation of COT1 in cis at Ser417 of the activation segment and interaction with MOB2, autonomous events that are independent of each other and are not regulated by the upstream germinal centre kinase POD6. Thr589 in the hydrophobic motif of COT1 is phosphorylated by POD6, further activating COT1 by inducing a conformational change through the interaction of the phosphorylated hydrophobic motif in the C-terminus with the hydrophobic pocket in the N-terminal lobe of the kinase. Intriguingly, Thr589 phosphoryation is not only important for maximal *in vitro* activity, but is also the critical signal for targeting the active kinase-MOB complex to the apical membrane of the hypha.

Longstanding evolutionary puzzles: Fungi as holders of the missing pieces. Kristiina Nygren, Anastasia Gioti and Hanna Johannesson. Uppsala University, Uppsala, Sweden, Hanna.Johannesson@ebc.uu.se

Evolutionary transitions are changes in adaptive traits that spread to replace ancestral conditions because they increase fitness. The driving forces, genetic mechanisms and genomic consequences of such transitions constitute puzzles for evolutionary biologists. The fungal kingdom exhibits a rich variety of reproductive strategies, and emerging genomic data from fungi could provide important insights into evolutionary transitions in reproductive mode. In this talk, I focus on theoretical predictions regarding the relationship between the mode of reproduction and genome evolution, and relate this to recent empirical data from Neurospora. According to theory, sexual inbreeding and asexuality are associated with smaller effective population sizes than sexual outbreeding, giving rise to reduced selection efficiency and genetic hitchhiking. This, in turn, is predicted to result in an accumulation of deleterious mutations and other genomic changes in selfing lineages. We used sequence information of multiple nuclear gene loci from 43 taxa to create a phylogeny of Neurospora, and gathered large-scale genomic data from four Neurospora lineages exhibiting an obligate selfing reproductive mode (homothallic species). The results suggest that transitions in reproductive mode from heterothallism to homothallism have occurred at least six times within this group of fungi, by different genetic mechanisms. Likelihood ratio tests indicate that reproductive mode is an important factor driving genome evolution in Neurospora. First, we found an increased ratio of nonsynonymous/synonymous substitution rates in homothallic branches as compared to heterothallics, suggesting a reduced efficiency of purifying selection in homothallic species. Furthermore, an elevated neutral substitution rate was found in heterothallic lineages as compared to the homothallic lineages. The latter finding is likely due to the presence of conidia in heterothallic species, i.e., a higher rate of mitotic divisions inducing mutations. One may speculate that the homothallic species of Neurospora have evolved a lower mutation rate to avoid genomic degeneration, possibly by switching off the conidial pathway. Taken together, we present a phylogenetic framework of Neurospora, opening up for in depth studies of transitions in reproductive behavior over evolutionary time.

Plenary Session IV: Regulatory Networks

Chair: Nancy Keller, University of Wisconsin, Madison

Mapping regulatory pathways using yeast functional genomics. Brenda Andrews, Karen Founk, Erin Styles, Lee Zamparo, Yolanda Chong, Zhaolei Zhang, Michael Costanzo and Charlie Boone. The Donnelly Centre, 160 College Street, University of Toronto, Toronto, ON, Canada M5S 3E1

To discover general principles of genetic networks and to define gene functional and biological pathways, our group has focused on the systematic identification of genetic interactions in the budding yeast. Synthetic genetic array (SGA) analysis provides a high throughput approach for systematic analysis of genetic interactions in budding yeast. We have used SGA analysis to construct a genome-scale genetic interaction map by examining 5.4 million gene-gene pairs for synthetic genetic interactions, generating quantitative genetic interaction profiles for about 75% of all genes in *Saccharomyces cerevisiae*. The global network identifies functional cross-connections between all bioprocesses including chromosome replication, repair and dynamics, mapping a cellular wiring diagram of pleiotropy.
We have also expanded our SGA platform to encompass other types of genetic interactions and to include cell biological phenotypes and quantitative read-outs of the activity of specific biological pathways. In one project, we have coupled synthetic SGA) technology with high-content screening (HCS) to detect subcellular morphology defects in yeast mutants. HCS enables virtually any pathway that can be monitored with a fluorescent reporter to be assessed quantitatively within the context of numerous genetic and environmental perturbations. As a proof-of-principle, we assessed DNA damage repair pathways by evaluating Rad52p-GFP foci in single mutants and a variety of genetically or chemically sensitized backgrounds. Computational analyses of single mutants alone using support vector machine-based classification revealed over 100 mutants exhibiting increased DNA damage foci, 60% of which were known to be sensitive to a variety of DNA damaging agents, validating our approach. Collectively, our experiments establish SGA-HCS as a powerful *in vivo* tool for revealing known and novel players of the DNA damage response in yeast. As we expand our method to include many more cellular compartments, we ultimately aim to provide an invaluable long-term resource of mutant subcellular morphology.

The evolution of gene regulation in *Ascomycota* fungi.
Aviv Regev and Dawn Anne Thompson, Broad Institute, MIT Department of Biology

Divergence of gene regulation is likely a major driving force in species evolution, but this hypothesis relies on a few specific examples and very general observations, because comparative functional studies are few and limited. In early studies, we traced the *cis*-regulatory evolution of gene modules, showing how alternative regulatory mechanisms evolve to perform the same function. However, functional information from few distant organisms is insufficient to decipher the evolution of regulatory mechanisms.More recently, our lab has developed an integrated experimental and computational system for comparative functional genomics of gene regulation. In this approach, we measure transcriptional responses and regulatory mechanisms across many species in a phylogeny, develop innovative algorithms to reconstruct the evolution of function, and use genetic manipulation to test the resulting model. We use 15 *Ascomycota* fungi, including *S. cerevisiae*, *S. pombe*, and *C. albicans*. Experimentally, we identified growth conditions for each species, and used species-specific microarrays, sequencing and metabolomics to measure mRNA, chromatin and metabolic profiles in each. Computationally, we developed Synergy, CladeoScope and Arboretum, algorithms that respectively chart the evolution of gene histories, cis-regulatory motifs, and the organization of regulatory gene modules.As a first demonstration, we studied the evolution of the ribosomal protein module in our system showing that an activator and a repressor that control RP gene regulation in *S. cerevisiae* were derived from the duplication and subsequent specialization of a single ancestral protein, and how another duplication led to sub-functionalization of the regulation of RP and RB genes. We also showed how loss of the derived repressor led to the loss of a stress-dependent repression in one species. We next tackled the mechanistic changes that explain differences in expression profiles in mid-log growth across all species. We measured chromatin organization and mRNA levels across 12 species, and discovered that chromatin organization at key gene modules – including carbon metabolism, mating, meiosis, and the peroxisome – has substantially diverged, consistent with changes in their regulation. This was mediated by changes in both intrinsic anti-nucleosomal sequences and *trans*-acting chromatin modifiers. We are now systematically reconstructing the regulation of expression levels following glucose depletion, showing the important role of sporadic gene duplications and whole genome duplications at presenting regulatory innovation in an other-wise well conserved response.

How oomycete and fungal effectors enter plant and animal cells. Brett M. Tyler[1], Shiv D. Kale[1], Vincenzo Antignani[1,2], Julio Vega-Arreguin[1], Ryan Anderson[1], Biao Gu[1,3], Daniel G. S. Capelluto[1], Daolong Dou[1], Emily Feldman[1], Amanda Rumore[1], Felipe D. Arredondo[1], Regina Hanlon[1], Jonathan Plett[4], Rajat Aggarwal[5] Isabelle Fudal[6], Thierry Rouxel[6], Francis Martin[4], Jeff J. Stuart[5] John McDowell[1], Christopher B. Lawrence[1], Weixing Shan[3]. [1] Virginia Polytechnic Institute and State University, Blacksburg, VA, USA; [2] University of Naples Federico II , Naples, Italy; [3] Northwest A & F University, Yangling, Shaanxi, China; [4] Centre INRA de Nancy, Champenoux, France; [5] Purdue University, West Lafayette, IN, USA; [6] INRA-Bioger, Campus AgroParisTech, Thiverval-Grignon, France.

Symbionts, both pathogenic and beneficial, must integrate their physiology with that of their host in order to achieve a successful colonization. Effector proteins that enter the cytoplasm of host cells are widely utilized for this purpose by bacterial, fungal, oomycete, protistan, nematode, and insect symbionts. While substantial progress has been made in understanding the molecular mechanisms of action of prokaryotic effectors, much less is known about the action of eukaryotic effectors, including the mechanisms by which they enter hosts cells. Oomycetes and fungi are destructive pathogens of a very wide range of hosts, including both plants and animals. The soybean pathogen *Phytophthora sojae*, one of the best characterized oomycete pathogens, encodes in its genome nearly 400 potential effector proteins with the cell-entry motif RXLR. We have recently identified the mechanism by which effector proteins from pathogens mutualists from two different kingdoms of life, Fungi and Oomycetes, enter the cells of their plant hosts [1]. Surprisingly, the same mechanism also enables those proteins to enter human cells. The mechanism involves the previously undetected presence of the phospholipid phosphatidylinositol-3-phosphate (PI-3-P) on the outer surface of the plasma membrane of both plant and human cells. The virulence proteins utilize PI-3-P as a receptor to gain entry via lipid-raft mediated endocytosis. More recently, we have discovered that two diverse insect pests of plants (hessian flies and aphids) also produce proteins that can bind PI-3-P via RXLR motifs in order to enter plant cells, where they suppress host defenses while the insects feed. Prompted by the observation of PI-3-P on human lung epithelial cells, we are currently exploring whether fungal pathogens that invade human lungs also utilize PI-3-P-binding virulence proteins. We are also exploring methodologies for disrupting PI-3-P-mediated effector entry in order to create new means for managing oomycete and fungal diseases and insect pests. 1. Kale SD, *et al.* 2010. External lipid PI3P mediates entry of eukaryotic pathogen effectors into plant and animal host cells. *Cell* 142: 284-295.

Regulatory Networks Governing Global Responses to Changes in Light and Time. Jay C. Dunlap, Department of Genetics, Dartmouth Medical School, Hanover, NH 03755

Free-living fungi live in a profoundly rhythmic environment characterized by daily changes in light intensity and temperature. Some fungi have well described systems for anticipation of temporal change, circadian systems, and nearly all fungi can respond acutely to changes in light intensity.

The nuts and bolts of the regulatory structures underlying circadian regulation and responses to blue light are well known in Neurospora. The circadian clock comprises a negative feedback loop wherein a heterodimer of proteins, WC-1 and WC-2, acts as a transcription factor (TF) to drive expression of *frq*. FRQ stably interacts with a putative RNA helicase (FRH) and with casein kinase 1, and the complex downregulates the White Collar Complex (WCC). With appropriate phosphorylation mediated delays, this feedback loop oscillates once per day (Baker, Loros, & Dunlap, FEMS Microbiol. Rev., in press). In turn, blue light is detected by FAD stably bound by WC-1, eliciting photochemistry that drives a conformational change in the WCC resulting in activation of gene expression from promoters bound by the WCC (Chen, Dunlap & Loros, FGB 47, 922-9, 2010).

With this as context, we** can now be at play in the genome using the tools of next generation sequencing, recombineering and luciferase reporters, finding out how the initial simple steps of clock control and light perception ramify via regulatory networks to command the genome to elicit development in response to the cues of light and time. Interestingly, the same players and networks appear to be involved in many places. For instance, the circadian feedback loop yields rhythmic activation of WCC, and genes encoding TFs that do not affect the feedback loop provide circadian output. The transcriptional repressor CSP-1 is one of a number of TFs induced within minutes by blue light (Chen et al. EMBO J. 2009) and it is also regulated by the circadian clock (Lambreghts et al. GENETICS 2007) with an mRNA peak in the late night and the CSP-1 protein following in the morning. ChIP-seq shows CSP-1, like WCC, binding to many regions of the genome to influence the expression of both light- and clock-controlled genes, but leading to peaks at times of day different from the spectrum of genes controlled by WCC. In this manner CSP-1 acts as a second order regulator, transducing regulation from light responses or the core circadian oscillator to a bank of output clock-controlled genes (ccgs), some of which are in turn other transcription factors. Assembling the global regulatory networks governing light and clock regulation is a privilege that will occupy us for a while.

**PIs: Jennifer Loros and members of the Program Project Team, “Functional Analysis and Systems Biology of Model Filamentous Fungi”, including the PIs Deb Bell-Pedersen, Kathy Borkovich, Michael Freitag, James Galagan, Heather Hood, Kevin McCluskey, Steve Osmani, Mike Plamann, Matt Sachs , Eric Selker, Jeff Townsend

Regulation of the compartmentalization of secondary metabolite biosynthesis. John E. Linz, Ludmila Roze, Anindya Chanda; Michigan State University

We previously demonstrated that vesicles play an important role in the synthesis, storage, and export of aflatoxin in *Aspergillus parasiticus*. As part of this work, we demonstrated that the late aflatoxin enzymes OmtA and OrdA are present and functional in this sub-cellular compartment; middle and early enzymes were also present but their function was not analyzed. To detect additional aflatoxin enzymes and other enzymes involved in secondary metabolism, we recently analyzed the proteome of a vesicle-vacuole (V) fraction purified from *A. parasiticus* grown under aflatoxin inducing and non-inducing conditions by multidimensional protein identification technology (MudPIT). We identified over 300 proteins associated with (V) fraction under both growth conditions. Of particular significance, we identified 8 aflatoxin enzymes with high reliability and 8 additional enzymes at lower reliability only under aflatoxin inducing conditions suggesting that the entire aflatoxin pathway may be present in vesicles. We also identified enzymes involved in synthesis of other secondary metabolites as well as catalase, superoxide dismutase, heat shock proteins, trehalose synthase, and xylulose synthase that are associated with fungal response to oxidative, thermal, and osmotic stress. These data suggest that vesicles are broadly involved in secondary metabolism and in mediating stress response. We are now analyzing the function of stress response and secondary metabolism enzymes in (V) fraction.

Recent data suggest that members of the bZIP family of transcriptional regulators (AtfB, AP-1) as well as a global regulator of secondary metabolism (VeA) play key roles in a regulatory network that coordinates the biogenesis of vesicles as well as the synthesis, localization, and the function of many enzymes contained in this sub-cellular compartment. This regulatory network appears to coordinate expression of secondary metabolism, stress response, and tethering complex genes (necessary for vesicle fusion to vacuoles). Identification of consensus binding sites in target gene promoters and the role of chromatin remodeling in this network are further topics for current work.

DOI: 10.4148/1941-4765.1066

Abstracts for Concurrent Session Talks

Comparative and Functional Genomics (Stajich/Thompson) **Merrill Hall**

Lascer capture microdissection, RNA-seq, and mutant genome sequencing: How to use next-generation sequencing to characterize developmental genes in filamentous fungi. Minou Nowrousian, Ines Teichert, Ulrich Kück Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Bochum, Germany; email minou.nowrousian@rub.de

Next-generation sequencing (NGS) techniques have revolutionized the field of genomics/functional genomics. We have recently sequenced and assembled the genome of the filamentous ascomycete *Sordaria macrospora*, a model organism for fungal development, solely from NGS reads (PLoS Genet 6:e1000891). We are now applying NGS in two approaches for the identification and characterization of developmental genes. (I) With laser capture microdissection, we can separate protoperithecia from the surrounding hyphae. RNA isolation and amplification from 150 protoperithecia yields enough material for RNA-seq analysis. The resulting data will be compared to RNA-seq data from whole mycelial exctracts to characterize the genome-wide spatial distribution of gene expression during sexual development. (II) We sequenced the genomes from two mutants that were generated by conventional mutagenesis, and identified two causative mutations through bioinformatics analysis. One mutant carries a mutation in the known developmental gene *pro41*. The second, a spore color mutant, has a point mutation in a gene that encodes an enzyme of the melanin biosynthesis pathway. For both mutants, transformation with a wild-type copy of the affected gene restored the wild-type phenotype. These data show that whole genome-sequencing of mutant strains is a rapid method for the identification of developmental genes.

Identification and potential function of natural antisense transcripts in the fungal plant pathogen *Ustilago maydis*. Michael E. Donaldson[1] and Barry J. Saville[1]. [1] Trent University, DNA Building, 2140 East Bank Dr., Peterborough, ON, K9J 7B8, Canada.

Natural antisense transcripts (NATs) corresponding to a number of open reading frames in the fungal plant pathogen *Ustilago maydis* were uncovered during the analysis of ESTs. Roles of NATs in regulating gene expression include: (1) transcriptional interference, (2) RNA masking, and (3) dsRNA-dependent mechanisms such as the broadly conserved RNA interference (RNAi) pathway. While plants, animals and most fungi contain functional RNAi machinery, phylogenetic and functional analyses have revealed that select yeast species and *U. maydis* do not. The role of NATs in *U. maydis* is currently unknown. We have characterized over 200 NATs by fully sequencing their corresponding antisense cDNAs. Using strand-specific RT-PCR, we determined that NATs are differentially expressed across a range of cell types, or expressed in a cell type-specific manner. The relationship between sense-antisense transcript pairs at four loci was examined in detail. In haploid cells, strand-specific quantitative-PCR, showed that at one of these four loci, the over-expression of antisense transcripts, whose expression naturally occurs in the dormant teliospore, increased the levels of its corresponding sense transcript. As a whole, experiments suggest that specific *U. maydis* antisense transcripts have the ability to stabilize sense transcripts. This action may be linked to the maintenance of mRNA integrity during teliospore dormancy and the controlled transition to actively translated mRNAs upon germination.

A method for accurate prediction of the size of secondary metabolite clusters in *Aspergillus nidulans*. Mikael R. Andersen[1], Jakob B. Nielsen[1], Mia Zachariassen[1], Tilde J. Hansen[1], Kristian F. Nielsen[1], and Uffe H. Mortensen[1]. [1]Center for Microbial Biotechnology, Technical University of Denmark, Denmark.

Fungal secondary metabolites (SMs) are receiving increasing interest due to their role as bioactives, ranging from antibiotics over cholesterol-lowering drugs to food toxins. The identification of SMs and their biosynthetic gene clusters are thus a major topic of interest. Identifying these genes is a tedious and time-consuming affair, with the standard method requiring the knockout of genes on both sides of putative SM synthases. Furthermore, one does not know the number of genes in the cluster and thereby extent of this work before starting the experiment. In this work, we present an algorithm for prediction of the size of SM clusters in *Aspergillus nidulans*. The method is based on an gene expression catalog of >60 transcriptome experiments, using a diverse set of strains, media, carbon sources, and solid/liquid cultivations. Furthermore, the method is independent of the quality of annotation. Application of the algorithm has allowed the accurate prediction of the number of included genes in well-characterized gene clusters. including the 25 genes of the sterigmatocystin cluster and the emericellamide cluster (4 genes). The method has provided strong predictions of unknown clusters, some of which we have verified experimentally and identified the corresponding metabolites.

Arboretum: Thinking about trees to cluster expression across species. Sushmita Roy[1,3], Dawn Anne Thompson[1], Jay Konieczka[1,5], Manolis Kellis[1,2], Aviv Regev[1,2,4]. [1]Broad Institute of MIT and Harvard, [2]MIT Biology, [3]MIT Computer Science and AI Lab, [4]HHMI Early Career Scientist, [5]FAS Center of Systems Biology, Harvard University

Advances in high-throughput functional genomics are generating massive amounts of transcriptional data for many species spanning millions of years of evolution. These data enable comparative genomics approaches to go beyond the primary sequence level to the functional level, and systematically relate the evolution of complex phenotypes to the rewiring of transcriptional regulatory networks. However, methods for comparative analysis of these data scale to only a handful of species at a time requiring the development of novel computational algorithms that can maximally interrogate these multi-species data, and improve our understanding of the role of regulatory networks in adaptive complexity. We developed a novel computational algorithm, Arboretum, that extends expression clustering, a canonical type of analysis of microarray data, to dozens of species.

Arboretum is based on a probabilistic model of the data that incorporates phylogenetic information encoded in both gene and species trees to identify modules, defined as clusters of co-expressed genes, that are conserved across various subsets of species. Arboretum infers the clusters of genes in extant species, as well as the ``hidden'' cluster assignment in the ancestral species. This unique ability of Arboretum allows us to reconstruct the evolutionary trajectories of individual genes, and modules.

We applied Arboretum to expression data of 15 *Ascomycota* species spanning more than 300 million years of evolution, measuring transcriptional response under carbon limitation. Arboretum identified five main expression profiles that were conserved across the all *Ascomycota* species measured: highly induced, medium induced, no-change, medium repressed and highly repressed genes. Genes in the highly induced cluster were involved in stress related processes, while those in the highly repressed cluster were enriched in ribosomal biogenesis. Examination of the ancestral and extant cluster assignment of mitochondrial genes recapitulated the known convergent evolution of up-regulated mitochondrial genes in respiro-fermentative species, and the respiratory state of the most ancient ancestor. Arboretum also identified many previously unknown evolutionarily coherent shifts in cluster assignment (expression pattern) of groups of orthologous genes. This coherence allows us to make predictions about the functions of unknown genes in *S. cerevisiae* and their orthologs in other species. Overall, these results are consistent with known information about transcriptional pattern and evolution of mitochondrial genes under carbon limitation, and provide new insights into the evolutionary patterns of other previously unstudied groups of genes. Applying Arboretum to transcriptional data from other environmental conditions across different sets of species will help dissect the mechanisms of regulatory network evolution and its effect on the adaptability and evolvability of organisms.

The genome sequence for *Eremothecium cymbalariae* establishes a link between the *S. cerevisiae* ancestor and the streamlined genome of *Ashbya gossypii*. Juergen Wendland and Andrea Walther, Carlsberg Laboratory, Yeast Biology, DK-2500 Valby, Copenhagen, Denmark; jww@crc.dk

E. cymbalariae is a close relative of *A. gossypii*. Both species are filamentous fungi that show bifurcational (Y-shaped) tip growth. In contrast to *A. gossypii*, *E. cymbalariae* generates an aerial mycelium with hyphae that form sporangia at their tips. *E. cymbalariae* spores lack appendices with which spores of *A. gossypii* stick together in bundles. To explore these differences on a genomic level we have established the complete genome sequence for *E. cymbalariae* using a 454 approach. We obtained a 40x coverage of the genome and with additional paired-end sequencing of fosmids and directed PCRs assembled the genome of app 9.6Mb into *E. cymbalariae's* 8 chromosomes in contrast to only 7 chromosomes in *A. gossypii*. We found orthologs of app. 4700 genes present in the yeast ancestor plus app 170 tRNAs. Most of the genes of *E. cymbalariae* are within blocks of synteny with the yeast ancestor. Strikingly the conservation of synteny is greater between *E. cymbalariae* and the ancestral yeast rather than to *A. gossypii*. At syntenic positions several homolgs to *S. cerevisiae* or e.g. *K. lactis* are present in the *E. cymbalariae* genome that are absent from *A. gossypii*. This indicates that the *E. cymbalariae* genome represents a preWGD genome with close ties to the ancestral yeast. During evolution several decisive changes occurred in the *A. gossypii* genome that affected for example the mating-type loci, the removal of a transposon, the condensation of intergenic regions, a strong increase in GC-content, and chromosomal rearrangements. We will present phenotypic comparisons of *E. cymbalariae* and *A. gossypii* as well as insights into genome evolution of the *Eremothecium* lineage.

Fungal genomes as seen through the lens of evolution. Toni Gabaldón. Centre for Genomic Regulation (CRG), UPF, Barcelona, Spain

With more than a hundred genomes already available and many more in the pipeline, fungi constitute an ideal dataset to inquire about processes underlying genome evolution. The application of phylogenetics at genome scales (phylogenomics) allows us to look at the evolution of genomes from the perspective of all of its genes. Here I will describe how the reconstruction and analysis of genome-wide collections of gene phylogenies (i.e. phylomes) has enabled us to address several evolutionary-related questions, including the robustness of the fungal species tree, the impact of inter-domain horizontal gene transfer in fungal evolution, the establishment of orthology and paralogy predictions, and the evolution across fungal lineages of particular metabolic pathways. Our analyses reveal that large topological variations across gene trees is largely neglected in multi-locus species tree reconstructions and may confound reconciliation-based orthology prediction methods. Processes such as gene duplication and horizontal gene transfer were found to be generally widespread, providing the substrate for the acquisition of key functional innovations. Fungal phylomes and derived orthology and paralogy predictions are accessible at http://phylomedb.org.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Comparative Analysis of Dermatophyte Genomes. Diego A. Martinez[1], Sarah Young[1], Qiandong Zeng[1], Dermatophyte Genome Consortium, Bruce Birren[1], Ted White[2*], and Christina Cuomo[1]. [1]Broad Institute of MIT and Harvard, Cambridge, MA
[2] Seattle Biomedical Research Institute, Seattle, WA *Current address, University of Missouri- Kansas City, School of Biological Sciences.

Dermatophytes are fungi that cause superficial infections in humans and animals and are the most common fungal infectious agents on the planet, with current treatment costs exceeding one half-billion dollars annually. Despite the common occurrence of the disease little is known at the molecular level about the fungi that cause dermatomycosis. To unravel the genetic basis of this disease we have sequenced five dermatophyte genomes including the most common human dermatophyte, Trychophyton rubrum, along with related species that show differences in host preference and mating competence. These genomes were compared to outgroups including dimorphic fungi and Aspergilli to identify changes in content specific to the Dermatophytes as well as individual species. The Dermatophyte genomes are smaller than the outgroups, ranging from 22.5 to 24.1 Mb; the largest genome (T. equinum) has a larger amount of repetitive elements. Using comparative methods, we updated the annotations of the five species based on conservation of gene structures. Between 8,523 and 8,915 genes were predicted in each genome; this is slightly smaller than the outgroup fungi. The core gene set conserved in all five genomes includes nearly 80% of the protein coding genes. Based on whole genome alignments, the genomes are highly syntenic, with a small number of rearrangements between Trichophyton and Microsporon species. Further analysis of differences between genomes may help identify genes important for the specific adaptation of each species including potential virulence factors.

Genome evolution in the Irish potato famine pathogen lineage. Sophien Kamoun, The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom.

Eukaryotic plant pathogens, such as oomycetes and fungi, cause highly destructive diseases that negatively impact commercial and subsistence agriculture worldwide. Many plant pathogen species, including those in the lineage of the Irish potato famine organism Phytophthora infestans, evolve by host jumps followed by adaptation and specialization on distinct hosts. However, the extent to which host jumps and host specialization impact genome evolution remains largely unknown. This talk will provide an update on our work on genome evolution in the P.
infestans clade 1c lineage. To determine the patterns and selective forces that shape sequence variation in this cluster of closely related plant pathogens, we and our collaborators resequenced several representative genomes of four sister species of P. infestans. This work revealed extremely uneven evolutionary rates across different parts of these pathogen genomes (a two-speed genome). Genes in low density and repeat-rich regions show markedly higher rates of copy number variation, presence/absence polymorphisms, and positive selection. These loci are also highly enriched in genes induced in planta, such as disease effectors, implicating host adaptation in genome evolution. These results demonstrate that highly dynamic genome compartments enriched in non-coding sequences underpin rapid gene evolution following host jumps.

Photobiology (Idnurm/Corrochano) **Chapel**

Molecular basis of photoconidiation in *Trichoderma atroviride*. Ulises Esquivel–Naranjo, Miguel Hernandez-Oñate, Enrique Ibarra-Laclette, and Alfredo Herrera-Estrella. Laboratorio Nacional de Genomica para la Biodiversidad, Centro de Investigación y de Estudios Avanzados Campus Guanajuato. Mexico. aherrera@langebio.cinvestav.mx

Trichoderma is used as a photomorphogenetic model due to its ability to conidiate upon exposure to light. In total darkness, *T. atroviride* grows indefinitely as a mycelium provided that nutrients are not limiting. However, a brief pulse of blue light given to a radially growing colony induces synchronous sporulation. Photoconidiation in *Trichoderma* is controlled by the orthologs of the *N. crassa* white-collar genes (*blr1* and *blr2*). Recently, we have applied high-throughput sequencing technology to the study of the *Trichoderma atroviride* transcriptome. We obtained RNA samples from the wild type strain grown in the dark or after exposure to a pulse of white or blue-light, as well as from a photoreceptor mutant (Δblr-1) exposed to white light. We identified over 300 light responsive genes, both induced and repressed, the majority of them Blr1 dependent. However, there is an important set of genes that is induced independently of this photoreceptor. Among the genes identified there are TFs, DNA-repair enzymes, and a set chaperons, including heat shock proteins, suggesting that light is perceived as a stress signal by *Trichoderma*. We have obtained gene disruption mutants of several of the transcription factors, and other key genes; resulting in mutants that do not photoconidiate, and mutants that do not require light conidiate.

Regulation of stomatal tropism and infection by light in *Cercospora zeae-maydis*. Hun Kim[1], John Ridenour[1], Larry Dunkle[2], and Burton Bluhm[1]. [1]Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA. [2]Crop Production & Pest Control Research Unit, USDA-ARS, Purdue University, West Lafayette, IN 47907, USA.

The fungal genus *Cercospora*, comprised of over 3,000 named species, is one of the most ubiquitous and destructive groups of plant pathogenic fungi, and incurs extensive damage on staple food crops throughout the world. In this study, the discovery that light was required for *C. zeae-maydis* to infect leaves led to the identification of the putative blue-light photoreceptor *CRP1*. Disrupting *CRP1* via homologous recombination revealed roles in multiple aspects of pathogenesis, including tropism of germ tubes to stomata, the formation of appressoria, conidiation, and the biosynthesis of phytotoxins. *CRP1* was also required for photoreactivation after lethal doses of UV exposure. Intriguingly, putative orthologs of *CRP1* are central regulators of circadian clocks in other filamentous fungi, raising the distinct possibility that *C. zeae-maydis* uses light as a key environmental input to coordinate pathogenesis with maize photoperiodic responses. This study identified a novel molecular mechanism underlying infection through stomata in a filamentous fungus, underscores the critical role light plays in pathogenesis in *C. zeae- maydis*, and highlights the tractability of the maize/*C. zeae-maydis* pathosystem as a model for examining infection via stomata and the integration of host and pathogen responses to photoperiod.

Characterization of *Mucor circinelloides* light-response mutants by high-throughput sequencing. Santiago Torres-Martínez, Eusebio Navarro and Victoriano Garre. Department of Genetics and Microbiology, Faculty of Biology, University of Murcia, Murcia 30071, Spain. storres@um.es

Light regulates developmental and physiological processes in a wide range of organisms, including fungi. Particularly, Zygomycete fungi have developed complex mechanisms to control the responses to light that await detailed characterization at molecular level. The basal fungus *Mucor circinelloides* is a good model for this purpose because its genome has been sequenced and several molecular tools are available for its manipulation. *Mucor*, like other Zygomycetes, has three *white collar-1* genes (*mcwc-1a*, *mcwc-1b* and *mcwc-1c*) that code for photoreceptor-like proteins. Analyses of knockout mutants suggest that each of these genes controls a specific response to light. Thus, *mcwc-1a* and *mcwc-1c* control phototropism and photocarotenogenesis, respectively. To identify new genes involved in regulation by light, a number of mutants showing either reduced carotene accumulation in the light or increased carotene accumulation have been isolated. Some of them present mutations on known structural and regulatory carotenogenic genes. High-throughput genome sequencing of others revealed the presence of non-conservative SNPs in 1 to 20 gene coding regions. Although some mutations map in genes of unknown function, others are in genes coding for proteins that may be involved in light transduction, such as a F-Box protein. Progress in the characterization of these genes in regulation by light will be shown.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Expanding the molecular clock network of *Neurospora crassa*. Maria Olmedo [1], Rachel Edgar [2], John O'Neill [2], Akhilesh Reddy [2] and Martha Merrow [1]. [1] Molecular Chronobiology, University of Groningen, The Netherlands. [2] Department of Clinical Neurosciences, Institute of Metabolic Science, University of Cambridge, UK.

Organisms from all phyla are exposed to environmental changes that stem from the highly predictable day-night cycle. The circadian clock allows organisms to anticipate these cyclic changes that occur due to the rotational movement of the Earth. The eukaryotic molecular clockwork is thought to be based on transcription/translation feedback loops although the proteins that comprise these loops are different across organisms. In *N. crassa*, this loop is made up of FRQ, WC 1 and WC 2, however a less robust form of rhythmic conidiation persists in the absence of these proteins. Furthermore, post-transcriptional and post-translational regulation of clock components is emerging as an additional clock mechanism with ancient evolutionary roots. The antioxidant enzyme Peroxiredoxin 6 (Prdx6) was previously shown to be subject to time dependent post translational modifications in mouse liver. The *N. crassa* genome contains a gene for a mitochondrial PEROXIREDOXIN that shows 60% similarity with the mouse Prdx6. We have investigated the contribution of *N. crassa* PRX to the fungal circadian clock

Light Sensitivity of First and Second Tier Clock-Controlled Genes in *Neurospora*. Gencer Sancar[1], Erik Malzahn[1], Stilianos Ciprianidis[1], Krisztina Káldi[2], Britta Brügger[1], Elan Gin[3], Thomas Hoefer[3], Axel Diernfellner[1], Tobias Schafmeier[1], and Michael Brunner[1]. [1] University of Heidelberg Biochemistry Center, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany; [2] Department of Physiology, Semmelweis University, POB 259 H-1444 Budapest, Hungary; [3]German Cancer Research Center and BioQuant Center, Modeling of Biological Systems , Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

Light responses and photoadaptation of *Neurospora* depend on the photosensory light-oxygen-voltage (LOV) domains of the circadian transcription factor White Collar Complex (WCC) and its negative regulator Vivid (VVD). We found that light triggers LOV-mediated dimerization of the WCC. VVD disrupts and inactivates the WCC homo-dimers by the competitive formation of WCC-VVD hetero-dimers, leading to photoadaptation. During the day, expression levels of VVD correlate with light intensity, allowing photoadaptation over several orders of magnitude. At night, previously synthesized VVD serves as a molecular memory of the brightness of the preceding day and suppresses responses to light cues of lower intensity, such as moonlight. The WCC activates morning-specific expression of the transcription repressor CSP1. Genes controlled by CSP1 are rhythmically expressed and peak in anti-phase to genes directly controlled by WCC). A negative feedback buffers the amplitude of CSP1-dependent oscillations with respect to the activity of WCC in light and dark.

Roles for CSP-1 in Light and Circadian Clock-Regulated Gene Expression. Nicole Knabe[1], Chandrashekara Mallappa[1], Kristina M. Smith[2], Jillian M. Emerson[1], Erin L. Bredeweg[2], Fei Yang[3], Deborah Bell-Pedersen[3], Matthew S. Sachs[3], Michael Freitag[2] and, Jay C. Dunlap[1]. [1]Department of Genetics, Dartmouth Medical School, Hanover, NH, [2]Program for Molecular and Cellular Biology, Department of Biochemistry and Biophysics, and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331; [3]Department of Biology and Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX

The csp-1 gene encodes a transcription factor. It is induced by blue light (Chen et al. EMBO J. 2009) and is also regulated by the circadian clock (Lambreghts et al. GENETICS 2007). Both the gene and the CSP-1 are expressed with peaks in morning, and using ChIP-sequencing we find CSP-1 to bind to many regions of the genome and to influence the expression of both light- and clock-controlled genes. In this manner CSP-1 acts as a second order clock regulator, serving to transduce clock regulation of gene expression from the core circadian oscillator to a bank of output clock- controlled genes (ccgs) as verified by ccg-luciferase gene fusions.

Light-dependent gene induction in *A. nidulans* requires release of the repressor LreA and binding of the activator FphA. Maren Hedtke, Julio Rodriguez-Romero and Reinhard Fischer Karlsruhe Institute of Technology, Dept. of Microbiology, Karlsruhe, Germany Maren.Hedtke@KIT.edu

Light serves as an important environmental signal to regulate development and metabolism in many fungi and has been studied to some detail in *N. crassa* and *A. nidulans*. *A. nidulans* develops mainly asexually in the light and sexually in the dark. The red-light sensor phytochrome (FphA) and the WC-1 homologue blue-light receptor LreA have been shown to mediate the light response in *A. nidulans* (1). There is evidence that both proteins form a light regulator complex (LRC). LreB (WC-2) and VeA are probably also components of this complex (2). Using ChIP and qRT PCR we show that HA-tagged FphA and LreA bind to the promoters of the *A. nidulans* homologues of *N. crassa con-10 (conJ)* and *ccg-1 (ccgA)*. *conJ* and *ccgA* are both induced during development but are also strongly upregulated after short exposure to light. Surprisingly we found LreA bound to the *conJ* and *ccgA* promoter only in the dark probably acting as a repressor. In contrast, FphA is recruited to the promoters after short illumination and seems to function as activator of transcription. These results suggest that the LRC is not a tight protein complex but rather transient and that light induction depends on derepression followed by induction through FphA. (1) Blumenstein A. et al., (2005) *Curr. Biol* 15(20):1833-8 (2) Purschwitz J., Müller S. & Fischer R., (2008) *Mol. Genet. Genomics* 18(4):255-9

Light Control of Fungal Development and Secondary Metabolism in *Aspergillus nidulans,* Gerhard H. Braus, Molekulare Mikrobiologie und Genetik, Georg-August-Universität Göttingen, D-37077 Göttingen, Germany; gbraus@gwdg.de

Differentiation and secondary metabolism are correlated processes in fungi that respond to various parameters including light. The heterotrimeric *velvet* complex VelB/VeA/LaeA (Bayram et al., 2008) and the eight subunit COP9 signalosome complex (Busch et al., 2007; Braus et al., 2010, Nahlik et al., 2010) are required for the sexual cycle resulting in the formation of the closed fruiting bodies (cleistothecia) of *A. nidulans*. VeA bridges VelB to LaeA which is required for secondary metabolism and for the formation of nursing Hülle cells. VelB is part of a second complex, VelB/VosA, which reduces asexual spore formation and is required for spore viability (Bayram et al., 2010). The current state of the work in the laboratory will be presented.

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DOI: 10.4148/1941-4765.1066

Fungal Effectors (Glijzen/Thomma) **Heather**

Fungal LysM effectors perturb chitin-triggered host immunity. Ronnie de Jonge[1], Anja Kombrink[1], Peter van Esse[1], Naoto Shibuya[2], Bart P.H.J. Thomma[1*] [1]Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PD Wageningen, The Netherlands; [2]Department of Life Sciences, Meiji University, Kawasaki, Kanagawa, Japan. *presenting author: bart.thomma@wur.nl

Cladosporium fulvum is a biotrophic fungal pathogen that causes leaf mould of tomato. The *in planta* abundantly secreted *C. fulvum* effector Ecp6 (for extracellular protein 6) acts as a potent virulence factor. The Ecp6 protein contains three lysin motifs (LysMs), protein domains that were also identified in plant cell surface receptors that activate host immunity upon perception of chitin oligosaccharide PAMPs, breakdown products of fungal cell walls that are released during plant invasion. Affinity precipitation assays showed that Ecp6 is a chitin-binding protein and three binding sites per molecule were detected. We found that Ecp6 does not protect fungal cell walls against hydrolysis by plant chitinases. Rather, Ecp6 appears to prevent the activation of chitin-triggered immunity through scavenging of chitin oligosaccharide PAMPs. Interestingly, homologues of Ecp6 were identified in many fungal species. These effectors are collectively referred to as LysM effectors. A number of LysM effectors from other fungal plant pathogens have been produced. Similar to Ecp6, most of these bind chitin and are able to suppress chitin-triggered immunity. However, in contrast to Ecp6, some LysM effectors protect fungal cell walls against chitinases, while others appear to have different substrates than chitin.

Pathogen effectors reveal a complex host immune network. Jim Beynon[1], Jens Steinbrenner[1], Susan Donovan[1], Laura Baxter[1], Mary Coates[1], Rebecca Allen[1], Georgina Fabro[4], M. Shahid Mukhtar[3], Anne-Ruxandra Carvunis[2], Matjia Dreze[2], Petra Epple[3],Jonathan Jones[4], Marc Vidal[2], Pascal Braun[2] and Jeff Dangl[3]. [1]Warwick Life Sciences and Systems Biology, Warwick University, Wellesbourne, Warwick, CV35 9EF, UK. [2]Centre for Cancer Systems Biology, Dana Faber Center, Boston, MA, USA. [3]Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. [4]The Sainsbury Laboratory, John Innes Centre, Norwich, NR4 7UH, UK.

Plants posses a basal immune system that successfully prevents invasion by many organisms by detecting conserved structures of potential pathogens. True pathogens suppress this immune system by delivering a suite of proteins, pathogenicity effectors, to the host cells. Understanding how these effectors allow successful invasion of the host is the major challenge of plant pathology today. We work on the biotrophic oomycete pathogen *Hyaloperonospora arabidopsidis* that causes downy mildew disease on Arabidopsis. To identify the effector complement of this pathogen we have sequenced the genome of *H. arabidopsidis*. This has revealed that there are potentially more than 130 effector proteins that could be delivered to the plant cell to enable biotrophic pathogenicity. This raises the question as to what are the host targets of these effectors? We have used a high-throughput yeast two hybrid approach to reveal a highly connected network of plant proteins targeted by the effectors.

***Magnaporthe oryzae* effector dynamics during invasion of living rice cells.** Barbara Valent[1], Chang Hyun Khang[1], Martha C. Giraldo[1], Mihwa Yi[1], Gloria Mosquera[2] and Melinda Dalby[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan, Kansas, U.S.A. [2]International Center for Tropical Agriculture (CIAT), Cali, Colombia. bvalent@ksu.edu

To cause blast disease, *Magnaporthe oryzae* sequentially invades living rice cells using intracellular invasive hyphae (IH) that are enclosed in host-derived extrainvasive-hyphal membrane. IH are initially filamentous hyphae and then switch into bulbous hyphae that proliferate in the host cell. The IH repeat this differentiation process in each subsequently colonized host cell. Hyphal cells that undergo the morphogenetic switch (the filamentous and first bulbous IH cells) are associated with the biotrophic interfacial complex (BIC), which forms at the filamentous hyphal tip and remains beside the first differentiated bulbous IH cell. Known avirulence effectors and most other biotrophy-associated-secreted (BAS) proteins accumulate in BICs. The strong correlation between preferential BIC localization and host translocation and new evidence that the BIC-associated bulbous IH cells are undergoing active exocytosis support our working hypothesis that BICs are a staging center for effector translocation into the host cytoplasm. So far, fluorescently-labeled versions of PWL2 and 26 additional BAS proteins localize to BICs and are translocated into the cytoplasm of invaded rice cells. Additionally, the effector PWL2 and 23 of the translocated BAS proteins move ahead into uninvaded neighbor cells, presumably to prepare these cells before invasion. Some translocated proteins naturally accumulate in the host nuclei and others accumulate where the IH crossed into neighboring cells. This talk will focus on current understanding of blast IH development and effector biology, and on effector sequences that mediate BIC accumulation and host translocation.

The superfamily of necrosis and ethylene-inducing peptide 1 (Nep1) like proteins (NLPs) harbors cytotoxic and non-cytotoxic, virulence-promoting members. T Nuernberger[1], I Kuefner[1], G Anderluh[2], C Oecking[1]; Steve Whisson[3], Bart Thomma[4], Guido van den Ackerveken[5]. [1]University of Tuebingen, Center for Plant Molecular Biology, Tuebingen, Germany; [2]University of Ljubljana, Department of Biology, Ljubljana, Slovenia; [3]Scottish Crop Research institute, Dundee, Scotland; [4]Wageningen University, Lab of Phytopathology, The Netherlands; [5]University of Utrecht, Department of Biology, The Netherlands. *presenting author: thorsten.nuernberger@zmbp.uni-tuebingen.de

Members of the superfamily of necrosis and ethylene-inducing peptide 1 (Nep1) like proteins (NLPs) are widely found in bacteria, fungi and oomycetes. A subset of these proteins causes leaf necrosis on dicot, but not on monocot plants. NLP cytotoxicity was shown to be crucial for microbial virulence and a necrotrophic lifestyle of the producing microbe. X-ray crystallography-based analyses of two microbial NLPs revealed substantial fold conservation of these proteins with cytolytic toxins produced by marine organisms (actinoporins). Actinoporins bind to animal host sphingomyelin prior to membrane pore formation and cytolysis. While plants do not produce sphingomyelins, we show that the target site for NLP toxins is of lipid nature and resides in the outer layer of the plasma membrane of dicot plants. Membrane binding and phytotoxicity requires the presence of a coordinately bound calcium cation within an electrophilic cavity on NLPs, suggesting that the plant docking site is negatively charged. In binding assays, NLPs preferentially bind to phosphorylated phosphatidylinositols (PIP), and incubation of NLPs with PIPs inhibits the cytotoxic activities of these proteins. Thus, NLP susceptibility of plant membranes is determined by its interaction with yet unknown PIP-like lipid structures that define a biologically significant difference in the composition of plasma membranes from monocot and dicot plants. Recently, the production by various oomycetes and fungi of non-cytotoxic members of the NLP superfamily was shown. The possible mode of action of these proteins, their biological activity as well as their contribution to microbial virulence will be discussed.

Crystal Structure Of The Avirulence Gene *AvrLm4-7* Of *Leptosphaeria maculans* Illuminates Its Evolutionary And Functional Characteristics. I. Fudal[1], F. Blaise[1], K. Blondeau[2], M. Graille[2], A. Labarde[2], A. Doizy[2], B.M. Tyler [3], S.D. Kale[3], G. Daverdin[1], M.H. Balesdent[1], H. van Tilbeurgh[2] and T. Rouxel[1] [1] INRA-Bioger, Grignon, France [2] IBBMC-CNRS / Université Paris-Sud, Orsay, France [3] Virginia Bioinformatics Institute, Blacksburg, USA

Leptosphaeria maculans, an ascomycete causing stem canker of oilseed rape, develops “gene-for-gene” interactions with its host plant where fungal avirulence (*AvrLm*) genes are the counterpart of plant resistance (*Rlm*) genes. *AvrLm4-7* encodes a 143 aa cysteine-rich protein, potentially secreted, strongly induced during primary leaf infection and involved in fungal fitness. AvrLm4-7 is translocated into plant and animal cells. This translocation is mediated by binding to PI-3-P and necessitates the presence of a RxLR-like motif. AvrLm4-7 crystal structure was determined following heterologous production in *Pichia pastoris*. The protein shows the presence of 4 disulfide bridges and is strongly positively charged, suggesting interaction with minus charged molecules (DNA, phospholipids). AvrLm4-7 confers a dual specificity of recognition by *Rlm7* or *Rlm4* resistance genes and occurs as three alleles only: the double avirulent (A4A7), the avirulent towards *Rlm7* (a4A7), or the double virulent (a4a7). A unique event of mutation, leading to the change of a glycine residue to an arginine, an amino acid located on an external loop of the protein, is responsible for the A4A7 to a4A7 phenotype change, strongly suggesting the importance of this protein region for recognition by the *Rlm4* gene, but not for the effector function of *AvrLm4- 7*.

The oomycete RxLR-effectors AVR3a and SpHtp1 show cell type specific import and their RxLR-leaders mediate dimerisation. Stephan Wawra[1], Severine Grouffaud[1,2], Judith Bain[1], Anja Matena[3], Claire Gachon[4], Irene de Bruijn[1], Stephen Whisson[2], Peter Bayer[3], Paul Birch[2], Pieter van West[1] [1] Aberdeen Oomycete Laboratory, Aberdeen (UK) [2] Scottish Crop Research Institute, Dundee (UK) [3] Universität Duisburg-Essen, Essen (Germany) [4] Scottish Association for Marine Science, Oban (UK)

The fungus-like oomycetes contain several species that are devastating pathogens of plants and animals. During infection oomycetes translocate effector proteins into host cells where they interfere with host defence responses. Several oomycete effectors have a conserved Arg-Xaa-Leu-Arg (RxLR)-motif that is important for their delivery. We found that, whereas the RxLR-leader sequence of SpHtp1 from the fish pathogen *Saprolegnia parasitica* shows fish cell- specific translocation, the RXLR-leader of AVR3a from the potato-late-blight pathogen *Phytophthora infestans* promotes efficient binding of the C-terminal effector domain to several cell types. Our results demonstrate that the RxLR- leaders of SpHtp1 and AVR3a are dimerisation sites, able to form heteromers. Furthermore, cell surface binding of both RxLR-proteins is mediated by an interaction with modified cell surface molecules. These results reveal a novel effector translocation route based on effector dimerisation and receptor modification, which could be highly relevant for a wide range of host-microbe interactions.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Symptom formation of *Sporisorium reilianum* on maize is mediated by secreted effectors. Hassan Ghareeb, Mohammad T. Habib, Yulei Zhao, Jan Schirawski Georg-August-Universität Göttingen, Albrecht-von-Haller Institute, Molecular Biology of Plant-Microbe Interactions, Untere Karspüle 2, 37073 Göttingen, Germany. jschira@uni-goettingen.de

Sporisorium reilianum and *Ustilago maydis* are closely related biotrophic pathogens of maize that cause different symptoms. Upon penetration of seedling plants by *S. reilianum*, fungal hyphae proliferate and spread throughout the plant initially without noticeable impact on plant health. Prominent symptoms are visible at flowering time, when spore-filled sori or leaf-like structures appear in the inflorescences. In addition, infected plants develop more female inflorescences than mock-treated plants. In contrast, an *Ustilago maydis* infection of maize leads to the formation of spore-filled tumors in the vicinity of the site of infection, which can occur on leaves, stems or flowers. To elucidate the molecular basis of the difference in symptom formation, the genome of *S. reilianum* was sequenced and compared to that of *U. maydis* [1]. Both genomes are highly syntenic and most encoded proteins are well conserved. However, a large region on chromosome 19 encoding more than 20 secreted effector proteins shows considerable divergence. We have dissected the contribution of the different fungal effectors of this region to symptom formation of *S. reilianum*. We show that different effectors are responsible for different aspects of the symptoms observed. We have identified one effector whose presence leads to an increase in the number of female inflorescences produced by the plant. However, effector deletion does not affect virulence of the strains. This shows that the different effectors located in the divergence region have a distinct contribution to symptom development of *S. reilianum*. [1] Schirawski et al., 2010. Science 330: 1546-1548.

Genome analysis of a strain from the UK blue 13 clonal lineage of *Phytophthora infestans* reveals significant genetic and expression polymorphisms in effector genes. Liliana M. Cano[1], Sylvain Raffaele[1], Ricardo Oliva[1], David Cooke[2], Paul Birch [2]and Sophien Kamoun[1]. [1] The Sainsbury Laboratory, JIC Norwich Research Park. NR47UH, Norwich, UK. [2] SCRI, Invergowrie, Dundee. DD25DA, Scotland, UK.

Phytophthora infestans is an oomycete pathogen that causes the devastating late blight disease in potatoes. In 2005, a clonal lineage of the A2 mating type, termed genotype blue 13, was identified in the UK and now this strain has become the most prevalent in the country. *P. infestans* blue 13 strains are characterized by an increased aggressiveness and virulence on several resistant potato varieties. Genome analysis of *P. infestans* blue 13 UK3928 strain revealed regions containing RXLRs with copy number variation (CNV) represented by increased depth of coverage. In addition, a whole-genome microarray screen allowed the detection of specifically induced genes on potato with no induction in the less virulent *P. infestans* reference strain T30-4. Our findings suggest that *P. infestans* blue 13 exhibit significant CNV and expression polymorphisms in effector genes. A better understanding of the genetic variation of *P. infestans* blue 13 will help to provide clues of the evolution of virulence of this epidemic disease.

Symbiosis (Zuccaro/Duplessis) **Fred Farr Forum**

The art and design of harmony: novel arbuscular mycorrhizal factors from cereals. Ruairidh Sawers, Caroline Gutjahr, Marina Nadal and Uta Paszkowski**.** Department of Plant Molecular Biology, The University of Lausanne, Switzerland

The mutualistic arbuscular mycorrhizal symbiosis occurs between roots of most land plants and fungi of the Glomeromycota. Mutualism is manifested in the bi-directional nutrient exchange where the plant provides photosynthates to the fungus and receives minerals, in particular phosphate, in return. Establishment of the symbiosis involves a pre-symbiotic molecular cross-talk that leads to recognition and subsequent hyphopodia formation on the root surface. The root epidermal cell underneath a hyphopodium prepares for the anticipated penetration by assembly of a prepenetration apparatus that guides the entering hypha through the cell lumen. Fungal growth continues from the outer cell layers towards the inner cortex of the root where intercellular proliferation along the longitudinal axes of the root permits rapid colonization of the root. In addition, the fungus develops highly branched hyphae, so called arbuscules, inside living cortex cells. These elaborate fungal haustoria dramatically change host cell architecture including the production of a extensive periarbuscular membrane that increases the surface area for nutrient exchange.
Over the past decade a number of plant encoded AM-factors were isolated that have provided a first glimpse into the nature and complexity of the molecular dialogue underpinning this apparently harmonious symbiosis. During my presentation I will introduce novel AM factors and candidates recently identified in my laboratory from cereals.

***Laccaria bicolor* aquaporins: functions in soil growing hyphae and ectomycorrhizal symbiosis.** Uwe Nehls[1,2], Sandra Dietz[2], Julia von Bülow[3], Eric Beitz[3], 1: University of Bremen, Faculty for Biology and Chemistry, Botany, Bremen, Germany 2: University of Tübingen, Microbiological Institute, Physiological Ecology of Plants, Tübingen, Germany 3: University of Kiel, Department of Pharmaceutical and Medicinal Chemistry, Pharmaceutical Institute, Kiel, Germany nehls@uni-bremen.de

Soil humidity and bulk water transport are essential for nutrient mobilization. Ectomycorrhizal fungi, bridging soil and fine roots of woody plants, are capable of modulating both by being integrated into water movement driven by plant transpiration and the nocturnal hydraulic lift. Aquaporins are integral membrane proteins that enable a concentration gradient driven flux of water and small uncharged ions over biological membranes. To gain insight into ectomycorrhizal fungal aquaporin function, we took advantage of the currently sequenced *Laccaria bicolor* genome. Here we present the first comprehensive study of a basidiomycotic aquaporin gene family, covering gene expression as well as protein function. Two aspects of aquaporin function were in focus of this investigation: water and solute permeability. While nearly all of the seven identified *L. bicolor* aquaporins mediated water permeability of *Xenopus laevis* oocyte plasma membranes, only three proteins revealed reasonable high rates for being of physiological significance. Protein function and gene expression data indicated these aquaporins to be mainly responsible for water permeability of fungal hyphae in soil and ectomycorrhizas. However, as growth temperature and ectomycorrhiza formation modified gene expression profiles of these aquaporins, specific roles in those aspects of fungal physiology are suggested. Moreover, two aquaporins, which were highly expressed in ectomycorrhizas, conferred plasma membrane ammonia permeability in yeast, pointing them towards being an integral part of ectomycorrhizal fungus-based plant nitrogen nutrition in symbiosis.

Identification of secreted *Glomus intraradices* signals activating the plant symbiotic program. Cristina Albarran, Hannah Kuhn and Natalia Requena Plant-Microbe Interactions, Karlsruhe Institute of Technology, Hertzstrasse 16, D-76187 Karlsruhe, Germany. cristina.albarran@bio.uka.de

Arbuscular mycorrhizal (AM) fungi form long-term symbiosis with roots of more than 80% of all land plants and are obligate biotrophs. Similar to other biotrophic fungi colonizing plants, AM fungi need to avoid the defense mechanisms of the plant to develop within the host. A way to achieve this is the delivery of diffusible fungal effectors molecules, termed Myc-factors, which initiate the symbiotic program even before both organisms contact. Although our understanding of the molecular dialogue between AM fungi-host has been improved in the recent years with some clues about the nature of the Myc-factors, still little is know and further investigation is required. In our group, it has been recently shown that some plant genes are specifically induced at early stages by diffusible signals produced by the fungus *Glomus intraradices*. While this activation is partially travelling through the symbiotic transduction pathway (SYM pathway) we have shown that a second cascade is required for the activation of some of those early genes. This suggests that possibly several Myc-factors are secreted at the same time by the fungus. We have established a reporter-assay for the identification and isolation of these Myc-factors. Furthermore, the use of SYM-mutant plant lines will allow distinguish each compound and the signalling cascade that leads to the activation of each gene. Research supported by AvH Foundation and DFG.

Genome expansion and gene loss in powdery mildew fungi reveal functional tradeoffs in extreme parasitism. Pietro Spanu and the BluGen sequencing Consortium. Imperial College London, UK and Others.

Powdery mildews are phytopathogens whose growth and reproduction are entirely dependent on living plant cells. The molecular basis of this lifestyle, obligate biotrophy, remains unknown. We present the genome analysis of barley powdery mildew, *Blumeria graminis* f.sp. *hordei (Blumeria)* , and a comparison with those of two powdery mildews pathogenic on dicotyledonous plants. These genomes display massive retrotransposon proliferation, genome size expansion and gene losses. The missing genes encode enzymes of primary and secondary metabolism, carbohydrate- active enzymes and transporters, probably reflecting their redundancy in an exclusively biotrophic lifestyle. Among the 248 candidate effectors of pathogenesis identified in the *Blumeria* genome very few (<10) define a core set conserved in all three mildews, suggesting that most effectors represent species-specific adaptations.

Genome and transcriptome analyses of *Piriformospora indica* provide hints into endophytic life strategies. A. Zuccaro[1,2], Lahrmann[1], Güldener[3], Pfiffi[1], Langen[2], Biedenkopf[2], Samans[2], Martin[4], Wong[3], Basiewicz[2], Murat[4], Kogel[2]. [1]Max-Planck-Institut für terrestrische Mikrobiologie Karl-von-Frisch-Straße 10 D-35043 Marburg, Germany. [2]Research Centre for Biosystems, Land Use, and Nutrition, Justus Liebig University, 35392 Giessen, Germany. [3]Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum, München, [4]Interactions Arbres/Micro-Organismes, INRA-Nancy, 54280 Champenoux, France

The multinucleate endophyte Piriformospora indica (Sebacinales, Basidiomycota) is an experimental model for mutualistic symbiosis with plants. The fungus colonizes the root cortex of a wide range of vascular plants promoting their growth and inducing resistance against abiotic and biotic stresses. Fungal development in roots combines colonization of dead cortex cells and biotrophic growth. These features together with its ability to grow readily on various synthetic media reveal substantial phenotypic plasticity which is reflected in its genomic traits. In the 25 megabase genome, 11,768 putative protein encoding genes were identified and these comprise clusters of multigene families with large expansion proposed to function in the regulation of cellular responses to stress and nutrient availability. These traits are shared by the ectomycorrhizal fungus Laccaria bicolor but not by the saprophytic fungus Coprinopsis cinerea. In contrast to ectomycorrhiza, cell wall degrading enzymes and peptidases are strongly expanded in P. indica and proved to be tightly regulated during symbiotic colonization. A P. indica specific domain expansion was observed for carbohydrate binding proteins. Some of these proteins resemble lectins and may function to mask ligands during host cell colonization. Gene loss in P. indica reveals tradeoffs towards biotrophic life-style. The missing genes encode enzymes of primary and secondary metabolism counting nitrate transporter and nitrate reductase, polyketide and nonribosomal peptide synthetases indicative of the non pathogenic character of P. indica. More than 300 small secreted proteins were identified, including a P. indica specific gene family of unknown function, several of which are strongly expressed during colonization of living roots. Similarities to ectomycorrhizal fungi during symbiosis were identified but cytological investigations, comparative analysis of the genomic traits and gene expression profiles during the early penetration phase identified substantial differences in the colonization strategy. This support the idea that biotrophic lifestyle in root systems arose independently through different functional specialization.

The transcription regulator ProA is essential for *Epichloë festucae*-perennial ryegrass symbiosis maintenance. Aiko Tanaka [1,2], Sanjay Saikia[2], Gemma Cartwright[2], Daigo Takemoto[1,2], Takashi Tsuge[1] and Barry Scott[2]. [1]Nagoya University, Nagoya Japan. [2]Massey University, Palmerston North, New Zealand. Email: d.b.scott@massey.ac.nz

The fungal endophyte, *Epichloë festucae*, forms a symbiotic association with perennial ryegrass, *Lolium perenne*. A genetic screen to identify *E. festucae* genes required for symbiotic maintenance has been carried out using *Agrobacterium* T-DNA mediated mutagenesis. The screen identified one mutant (Ag413) that causes severe stunting of the grass host, an interaction phenotype similar to that observed for a *noxA* deletion mutant (Tanaka et al. 2006). Sequence analysis of the T-DNA junction sequences in Ag413 showed that the T-DNA was inserted into a gene, designated *proA*, encoding a Zn(II) $_2$Cys$_6$ transcription factor. ProA has homology to Pro1/NosA, positive regulators of sexual development in other ascomycetes. Deletion analysis confirmed that stunting of the host plant is caused by disruption of *proA*. To identify the gene targets for ProA, we have analysed a publicly available microarray data set of a *pro1* mutant in *Sordaria macrospora* (Nowrousian et al. 2007) and selected 12 candidate genes that are down-regulated in *pro1*, and screened expression of the *E. festucae* homologues by qRT-PCR analysis. One gene, named *esdC*, was significantly down-regulated in the ryegrass/*proA* mutant interaction. *esdC* has been shown to be responsible for sexual development in *Aspergillus nidulans*. To determine whether ProA binds the *esdC* promoter directly, we performed electrophoretic mobility shift assays (EMSAs) using a purified MBP (maltose-binding-protein)-ProA 1-145aa fusion protein (MBP-ProA 1-145). The EMSA experiments showed direct binding of MBP-ProA 1-145 to a 25-bp region within the *esdC* promoter. Tanaka et al. (2006) Plant Cell 18, 1052-1066. Nowrousian et al. (2007) Mol. Microbiol. 64, 927-937.

Metabolomics meets Genomics: Solving the puzzle of how multiple cyclic oligopeptides are synthesised by epichloae endophytes via a single ribosomally encoded gene, *gigA*. Linda J Johnson, Albert Koulman, Geoffrey Lane, Karl Fraser, Christine Voisey, Jennifer Pratt, Gregory Bryan and Richard D Johnson. AgResearch, Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand. Phone: +64 6 351 8090. Fax: 15 +64 6 351 8032. Email: richard.johnson@agresearch.co.nz

Epichloae endophytes live symptomlessly within the intercellular spaces of cool-season grasses, and confer a number of biotic and abiotic advantages to their hosts. Metabolomics analysis, by direct infusion mass spectrometry, of the *Epichloë festucae*-perennial ryegrass association identified a new class of endophyte derived compounds (multiple cyclic oligopeptides) only in the guttation fluid of infected plants. In a parallel programme to knock out the most highly expressed endophyte gene during symbiosis, *gigA*, we found that all cyclic oligopeptides (COPs) were eliminated in the delta *gigA* mutant. Initial analysis of the predicted GigA protein, however, suggested that COPs were not a direct product of the gene. Here we describe how multiple COPs are synthesised via a single ribosomally encoded gene, *gigA*, which is expressed preferentially *in planta* and is one of the most abundantly expressed fungal transcripts in endophyte infected grasses. The GigA protein contains an N-terminal signal sequence and imperfect 27 amino acid repeats which we propose are processed by a kexin protease to yield multiple COPs of 8 or 9 amino acids. Deletion of *gigA*, and re-introduction of the mutant into the host plant, leads to complete loss of COP production, altered hyphal ultrastructure and an increase in fungal biomass. Recent evidence suggests that *gigA* forms part of a gene cluster with a kexin protease and two hypothetical proteins. This is the first report of multiple cyclic peptides being ribosomally encoded from a single gene and we are interested in both the mechanism of cyclisation and the function of these COPs in endophyte-grass symbioses.

Deconvoluting the *Neotyphodium coenophialum* genome. Carolyn Young[1], Ranamalie Amarasinghe[1], Johanna Takach[1], Patrick Zhao[1], Jennifer S. Webb[2], Neil Moore[2], Jolanta Jaromczyk[2], Charles T. Bullock[2], Jerzy W. Jaromczyk[2], Christopher L. Schardl[2]. [1]The Samuel Roberts Noble Foundation, Ardmore, Oklahoma; [2]University of Kentucky, Lexington Kentucky.

The epichloë endophyte *Neotyphodium coenophialum* forms a mutualistic association with the cool-season grass, tall fescue (*Lolium arundinaceum*). The wide range of benefits the endophyte provides its host has made tall fescue an agriculturally important grass. Unfortunately, some isolates are known to cause toxicity to grazing livestock due to the production of ergot alkaloids. To unravel the complexity of the endophyte-grass association, we have embarked on sequencing the *N. coenophialum* genome and identifying symbiosis induced genes. *N. coenophialum* is considered an interspecific hybrid consisting of origins from *E. festucae*, *E. typhina* and a *Lolium*-associated endophyte closely related to *E. baconii*, with an estimated genome size of 57Mb. We have started genome sequencing using cosmid/fosmid end sequencing and '454' shot-gun and paired end reads. Initial assembly of the 454 generated data indicated a larger genome size (95 Mb) than originally estimated. Transcriptome comparison using Illumina sequencing of mRNA from in planta vs in culture conditions showed some of the most highly expressed genes were those required for production of lolines and ergot alkaloids. Laser capture microscopy will be used to dissect the endophyte from the host to enrich for endophyte transcripts in planta and will be compared with the Illumina data. Deconvolution of the *N. coenophialum* genome will provide insight into the impact of repetitive elements in genome evolution and allow us to see what gene families, including those of secondary metabolism, have been retained or lost during hybridization.

Evolution of Sex in Fungi (Dyer/Debuchy) **Kiln**

Sex in basal fungi: ***Phycomyces, Mucor,* and *Rhizopus.*** Joseph Heitman[1], Soo Chan Lee[1], Charles H. Li[1], Alexander Idnurm[2], Maria Cervantes[3], Rosa M. Ruiz-Vazquez[3], Santiago R. Torres-Martinez[3], Andrii P. Gryganskyi[4], and Rytas Vilgalys[4]. [1]Department of Molecular Genetics and Microbiology, [4]Department of Biology, Duke University, [2]Division of Cell Biology and Biophysics, University of Missouri, Kansas City, and [3]Departamento de Genetica y Microbiologia, Universidad de Murcia, Spain

How sexual identity is defined and the roles of sexual reproduction in the generation of diversity and evolution are of general interest. Given its ubiquity, sex is thought to have arisen once early in the evolution of eukaryotes. The fungal kingdom provides a broad window on the evolution of sex and its myriad diversifications, and the close alignment of the fungal and animal kingdoms within the opisthokont supergroup of eukaryotes suggests that principles that emerge from studies of fungi will further advance our understanding of both kingdoms and beyond. Many fungi once thought to be asexual have been revealed by genomics to retain the machinery for sex, and laboratory studies are uncovering extant sexual cycles. In turn, the genomes of fungi with well-established sexual cycles provide insights on the mechanisms of sex determination and sexual reproduction. Molecular and genetic studies of the *sex*/mating type locus of model and pathogenic zygomycete fungi (*Phycomyces*, *Mucor*, *Rhizopus*) reveal divergent HMG domain proteins (SexM, SexP) define sexual identity and an interesting example of conidia spore size dimorphism linked to virulence. Comparisons of these species highlight the evolutionary trajectory of the *sex* locus, and suggest that HMG factors may be the ancestral sex determinant given their roles in sex determination in the *Mucorales*, hemi- and euascomycete species, and as the mammalian sex determinant Sry. Alternatively, there may have been two ancestral sex determining systems, one based on HMG factors and the other on homeodomain (HD) proteins, which have vied for pre-eminence during fungal evolution. Interestingly, in some extant fungal species both HMG and HD factors are encoded by the mating type locus. Taken together, these studies of sex and its determination, evolution, and impact throughout the fungal kingdom illustrate general principles by which genetic diversity is generated and maintained in eukaryotic microbes, with implications for both other fungal phyla and metazoans.

Tracing the origin of the fungal alpha1 domain places its ancestor in the HMG-box superfamily. Tom, Martin [1]#, Shun-Wen, Lu [2]#, Herman, van Tilbeurgh [3], Daniel, R. Ripoll [2], Christina, Dixelius[1], B. Gillian, Turgeon [2], Robert, Debuchy [3] [1]SLU, Uppsala, Sweden [2]Cornell University, Ithaca, USA [3]Univ Paris-Sud, Orsay, France # Equal contribution

Fungal mating types in self-incompatible Pezizomycotina are specified by one of two alternate sequences occupying the same locus on corresponding chromosomes. One sequence is characterized by a gene encoding an HMG protein, while the other, a gene encoding a protein with an alpha1 domain. DNA-binding HMG proteins are well characterized. In contrast, alpha1 domain proteins evolutionary origin is obscure, precluding a complete understanding of mating-type evolution in Ascomycota. alpha1 proteins have not yet been placed in any of the large families of sequence-specific DNA-binding proteins. We present sequence comparisons, phylogenetic analyses, and *in silico* predictions of secondary and tertiary structures, which support our hypothesis that the alpha1 domain is related to the HMG domain. We have also characterized a new conserved motif in alpha1 proteins of Pezizomycotina. This motif is downstream of the alpha1 domain and consists of a core sequence Y-[LMIF]-x(3)-G-[WL] in a larger conserved motif. Our data suggest that extant alpha1-box genes originated from an ancestral HMG gene, which confirms the current model of mating-type evolution within the fungal kingdom. We propose to incorporate alpha1 proteins in a new subclass of HMG proteins termed MATalpha_HMG.

Unusual features of the Botrytis cinerea mating system. Jan A.L. van Kan (1), Paul S. Dyer (2) and Linda M. Kohn (3) 1 Laboratory of Phytopathology, Wageningen University, The Netherlands, 2 School of Biology, University of Nottingham, United Kingdom; 3 Department of Ecology and Evolutionary Biology, University of Toronto, Canada. E-mail: jan.vankan@wur.nl

Botrytis cinerea is a heterothallic ascomycete with two mating types, MAT1-1 and MAT1-2. Fragments of the MAT1- 2-1 and MAT1-1-1 genes were detected bordering idiomorphs of MAT1-1 and MAT1-2 isolates, respectively. Both these fragments encode truncated, non-functional proteins. *B. cinerea* has probably evolved from a homothallic ancestor containing all genes, with MAT1-1 and MAT1-2 arising from the loss of HMG and alpha-domain sequences, leaving the disabled gene fragments present in current loci. Two ORFs, designated MAT1-1-5 and MAT1-2-3, have not previously been reported from other fungi. In a cross of a MAT1-1-5 knockout mutant with a wild type MAT1-2 strain, the stipe develops normally but transition to the differentiation of a cup is blocked. Most *B. cinerea* isolates act in a standard heterothallic fashion, but some isolates can mate with both MAT1-1 and MAT1-2 isolates and are referred to as 'dual maters'. Some dual mater isolates can self-fertilize and are truly homothallic. The MAT locus of five dual mater isolates was analysed. Four of those contain a MAT1-2 locus, without any part of the MAT1-1 locus being detected, whereas one homothallic isolate contains a MAT1-1 locus, without any part of the MAT1-2 locus being detected. We conclude that dual mating and homothallism are controlled by factors other than the MAT locus.

Fitness associated sexual reproduction in *Aspergillus nidulans.* Sijmen Schoustra. Wageningen University, the Netherlands

Sex is a long-standing evolutionary enigma. Although the majority of eukaryotes reproduce sexually at least sometimes, the evolution of sex from an asexual ancestor has been difficult to explain because it requires sexually reproducing lineages to overcome the manifold costs of sex, including the destruction of favorable gene combinations created by selection. Conditions for the evolution of sex are much broader if individuals can reproduce either sexually or asexually (i.e. facultative sex) and allocate disproportionately more resources to sex when their fitness is low. I will present results of a study using *Aspergillus nidulans* in a reciprocal transplant experiment across three environments. The results provide evidence for the existence of fitness associated sexual reproduction, demonstrating that allocation to sexual reproduction is a function of how well adapted a genotype is to its environment.

Fungal developmental networks: Control of fruiting body formation in *Sordaria macrospora.* Ines Teichert & Ulrich Kück Department for General and Molecular Botany, Ruhr-University Bochum, Universitaetsstrasse 150, 44780 Bochum, Germany, ines.teichert@rub.de

Fruiting body formation in filamentous ascomycetes is a complex differentiation process applicable as model for eukaryotic cell differentiation in general. Regulation of fruiting body formation involves a plethora of factors ranging from signaling components to transcription factors and metabolic enzymes, and is still not completely understood. In our studies, the homothallic ascomycete *Sordaria macrospora* serves as experimental system to gain a better understanding of the molecular mechanisms underlying fruiting body development. By complementation of sterile pro mutants, several proteins were identified that are essential for completion of the sexual life cycle. Since different PRO proteins localize to different compartments, protein-protein interaction studies were employed to link these proteins and to identify novel regulators of the sexual life cycle. Yeast-two hybrid and biochemical analyses hint to an extensive network regulating cellular differentiation in a fungal model system.

Sex-specific gene expression during asexual development of *Neurospora crassa* under constant light. Zheng Wang[1], Koryu Kin[1], Francesc Lopez-Giraldez[1], Hanna Johannesson[2], and Jeffrey P. Townsend[1]*. [1]Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect Street, New Haven, CT06520, USA. [2]Department of Evolutionary Biology, Uppsala University, Sweden.

The asexual growth and propagation are dominant in the life history of most filamentous fungi. Nevertheless, sexual reproduction reassorts significant genetic diversity within many fungal populations. Environmental factors such as light and nutrients affect asexual and sexual development differently in fungi. Nevertheless, homologous asexual and sexual reproductive structures observed in some ascomycetes suggest that shared genetic mechanisms underly the two types of development. While sex-determination loci play important roles in sexual development in fungi, it is widely assumed that sex-determination loci have little impact upon the dominant asexual stage in the life history of fungi. We investigated differences between mating types during asexual development with mRNA sampled from largely isogenic *mat A* and mat a *N. crassa* strains at early, middle, and late clonal stages of development under a condition of constant light. Mating-type genes, pheromone precursor and receptor genes were assayed with real-time PCR. Mating type genes were increasingly expressed during asexual development, and expression of pheromone precursors *ccg-4* and *mfa-1* and receptors *pre-1* and *pre-2* were detected in both mating types in all development stages. Gene expression for both mating types throughout vegetative development were characterized with a genome wide microarray analysis for developmental markers such as transcription factors, for genes related to conidiation, internal clock, cell division cycle, heat shock, and for light responsive genes. We observed significant differences in overall gene expression between the strains of different mating types across clonal development, especially at late development stages. In particular, the *mat A* genotype showed a higher expression level than *mat a* for numerous genes, and demonstrated greater transcriptional regulatory activity. In both mating types, significant up-regulation of expression of late light responsive genes was observed for late asexual development stages. Further investigation of the impact of light and the roles of light response genes in asexual development of both mating types is warranted

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Regulation of the Meiotic Program in *Candida lusitaniae*. R.K. Sherwood,[1,2] S. Torres[1] and R.J. Bennett. [1,2 1]Molec. Microbiology and Immunology. [2]Molec. Bio., Cell. Bio., and Biochemistry, Brown University, Providence, RI. Racquel_Sherwood@Brown.edu

Many genes involved in mating and meiosis are conserved across hemiascomycete yeast, including model species *S.cerevisiae*, as well as members of the *Candida* yeast clade. Completion of the mating cycle in sexual species is mediated by meiosis, in which reductive DNA division gives rise to recombinant progeny cells. Recent studies show that *C.lusitaniae* is unusual among *Candida* species in that it undergoes a complete sexual cycle, despite lacking homologs of several genes essential for meiosis in *S.cerevisiae*. In particular, *IME1*, encoding the master meiotic regulator in *S. cerevisiae* is absent from the *C.lusitaniae* genome. In this study, we use genetic and genomic approaches to identify regulators of meiosis in *C.lusitaniae*. We show that homologs of *S. cerevisiae* meiotic genes are induced during *C.lusitaniae* meiosis, suggesting these genes have retained a conserved function. We also constructed mutant strains lacking the serine threonine kinase, *IME2*, in *C.lusitaniae*. Preliminary experiments indicate that *IME2* plays an important role in multiple aspects of sexual reproduction in this species. Overall, we propose that elucidation of conserved and novel meiotic regulators in *C.lusitaniae* will provide further clues as to how different *Candida* species undergo meiosis, despite lacking factors essential to *S.cerevisiae* meiosis.

Regulation of mushroom development. Robin A. Ohm, Jan F. de Jong, Luis G. Lugones and Han A. B. Wösten. Department of Microbiology and Kluyver Centre for Genomics of Industrial Fermentations, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Mushrooms are an important food source and they produce molecules with therapeutic activities and enzymes that can be used for bioconversions. Moreover, they have been identified as promising cell factories for the production of pharmaceutical proteins. Many mushroom-forming fungi cannot be cultured in the lab nor genetically modified. The basidiomycete *Schizophyllum commune* is among the exceptions. In fact, it is the only mushroom-forming fungus in which genes have been inactivated by homologous recombination. Moreover, the genome of *S. commune* has been sequenced. The genome of *S. commune* revealed 472 genes that are predicted to encode transcription factors. Seven of these genes have been deleted. This resulted in the absence of mushroom development (in the case of deletion of *fst3*, *bri1* and *hom2*), in arrested development at the stage of aggregate formation (in the case of c2h2) and in the formation of more but smaller mushrooms (in the case of *fst4*, *hom1* and *gat1*). Moreover, it was shown that strains in which *hom2* and *bri1* were inactivated formed symmetrical colonies instead of irregular colonies like the wild-type. A genome-wide expression analysis identified several gene classes that were differentially expressed in the strains in which *hom2* or *fst4* were inactivated. Among the genes that were down-regulated in these strains were *c2h2* and *hom1*. Based on these results, a regulatory model of mushroom development is proposed. This model probably also applies to other mushroom forming fungi and will serve as a basis to understand mushroom formation in nature and to enable and improve commercial mushroom production. The latter will be facilitated by an inducible promoter system based on a gene encoding a heat shock protein. Using this system we were able to activate mushroom formation in *S. commune* by exposing this basidiomycete to a heat shock.

Evolution of Centromeres (Sanyal/Berman) **Nautilus**

Tracing the path of centromere evolution in yeasts. Kaustuv Sanyal[1], Jitendra Thakur[1], Gautam Chatterjee[1], Yogitha Tattikota[1], and Rahul Siddharthan[2]. [1]Molecular Mycology Laboratory, Molecular Biology & Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore 560064, India; [2]Institute for Mathematical Science, Taramani, Chennai, India sanyal@jncasr.ac.in

Faithful chromosome segregation requires an accurate attachment of the spindle microtubules (MTs) to the kinetochore (KT), a complex of proteins that is assembled on the centromere. The structure of the centromere and the number of MTs that attach to the KT varies among different fungal species. Only one MT attaches to a KT in the budding yeast *Saccharomyces cerevisiae*, which has a 125 bp short "point" centromere, while at least 2 -3 MTs attach to a KT in the fission yeast *Schizosaccharomyces pombe*, carrying a longer (40 – 110 kb) "regional" centromere. In addition, it has been observed that the components of the ten protein Dam1p complex (comprising the outer kinetochore that directly interacts with the spindle MTs) are essential in *S. cerevisiae*, but are dispensable for viability in *S. pombe*. This suggests that there has been a gradual change in both the centromere organization and the requirement for KT-MT interaction as these yeasts diverged from each other. *Candida* species, phylogenetically placed in between *S. cerevisiae* and *S. pombe*, are excellent systems to trace the path of this evolution. To this end, we first determined the centromere structure of the two closely related species, *Candida albicans* and *C. dubliniensis*, and found them to be of an "intermediate" type, with each centromere being unique and different on each chromosome. By contrast, we have recently discovered that the centromeres of another Candida sp., *Candida tropicalis*, are very similar to those of fission yeast. Thus, we can trace the evolution of centromeric DNA regions from simple point centromeres to more complex regional centromeres within the hemiascomycetes yeasts. In keeping with this, a comparison between the centromere DNA sequences of *C. albicans* and *C. dubliniensis* showed that these are probably the most rapidly evolving loci in their genomes. The protein machinery that is involved in KT–MT interaction (such as the Dam1 complex) appears to be keeping pace with this evolution of the centromere DNA as well. Deletion of some of the members of the Dam1p complex in *C. albicans* (~ 1 MT/KT) is lethal. All of these facts point to a potential co-evolution of centromere organization and the mechanism of KT-MT interaction in yeasts, which in turn may play an important role in speciation.

Centromeres in filamentous fungi. Kristina M. Smith[1], Pallavi Phatale[2], Christopher M. Sullivan[4], Kyle R. Pomraning[3], Lanelle Connolly[1] and Michael Freitag[1,2,3,4 1]Department of Biochemistry and Biophysics, [2]Department of Botany and Plant Pathology, [3]Program for Molecular and Cell Biology, [4]Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331, USA.

Centromeres serve as the foundation for kinetochore assembly and properly maintained centromeres are essential for attachment of spindle microtubules, which transport chromosomes into daughter nuclei during nuclear division. Over the past ten years little new information has emerged on the centromere organization in filamentous fungi. To date, only *Neurospora crassa* has been studied in any detail, enabled by groundbreaking studies on the underlying centromeric DNA structure carried out in the 1990s [1, 2] and the availability of an arsenal of genetic, biochemical and cytological tools to study centromere proteins and centromere DNA composition. We analyzed the Neurospora genome for the presence of satellite or other near-repetitive sequences but found instead that what has been found in earlier studies [2] holds true: Neurospora centromeric DNA is composed of relics of transposable elements that have undergone RIP. The near-repeat structure of RIPed DNA allows almost complete assembly of centromeric DNA, something that is difficult in other eukaryotes with large regional centromeres composed of several hundred kilobases of repetitive DNA sequences. To learn more about centromere assembly and maintenance, we subjected *Neurospora crassa* and *Fusarium graminearum* to ChIP-sequencing with tagged CenH3 and CenpC as well as antibodies against histone modifications thought to be required for centromere function. In Neurospora, we found colocalization of CenH3, CenpC and H3K9me3 in a 150-300 kb region on each chromosome. H3K4me2 was not enriched at Neurospora centromeres, in contrast to results from studies with plant, fission yeast, Drosophila and human core centromeric regions. DNA methylation was tightly associated with H3K9me3 and was enriched at centromere peripheries and overlapped little with Cen protein distribution. Mutation of *dim-5*, which encodes an H3K9 methyltransferase (DIM-5), and *hpo*, which encodes HP1, the chromo domain protein that binds H3 K9me3, resulted in partial loss of CenH3-GFP binding, mostly from the edges of the centromere regions. Our findings suggest that centromere maintenance in Neurospora is qualitatively different from that in fission yeast, where expression of small RNA and subsequent heterochromatin formation is required for the assembly but not maintenance of centromeres. [1] M. Centola and J. Carbon, 1994. Mol. Cell. Biol. 14:1510-1519. [2] E. Cambareri et al., 1998 Mol. Cell. Biol. 18:5465-77.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Genetic analyses of centromere-specific histone H3 proteins from three ascomycetes in *Neurospora crassa*. Pallavi Phatale, Kristina M. Smith and Michael Freitag. Department of Botany and Plant Pathology, Department of Biochemistry and Biophysics, and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

Centromere assembly and inheritance are dynamic and organism-specific. Protein complexes involved in kinetochore assembly contain signature proteins that are highly conserved in most eukaryotes, while other proteins, or certain domains, are divergent even between strains within one taxon. This predicts the existence of both conserved as well as divergent protein interactions during centromere and kinetochore assembly and maintenance. The "centromere identifier", a centromere-specific histone H3 (CenH3) forms the platform for centromere assembly and is one of these bipartite proteins. It contains a hypervariable N-terminal region and a highly conserved histone fold domain (HFD). We previously showed that C-terminally tagged *Podospora anserina* CenH3 (PaCenH3-GFP) substitutes for Neurospora CenH3 (NcCenH3) in mitosis and meiosis. Replacement of NcCenH3 with *Fusarium graminearum* CenH3 (FgCenH3) supported only mitosis in Neurospora and tagging at the C-terminus resulted in defects in meiosis. Domain swapping experiments of the N-terminus of FgCenH3 with the HFD of PaCenH3 allows mitosis and meiosis, but chimeras with N-terminal NcCenH3 or PaCenH3 combined with the HFD domain of FgCenH3 were infertile or barren. Results from domain-swapping experiments suggest that only a few amino acids within the HFD are crucial during meiosis. There are only 16 differences between PaCenH3 and NcCenH3 in the HFD region. We propose that these differences play an important role during the assembly and inheritance of regional centromeres.

Outside looking in – A view of the centromere architecture from the kinetochore. Ajit Joglekar, Cell & Developmental Biology, University of Michigan Medical School

The three dimensional architecture of the centromere and its establishment is an extremely complex subject matter that is equally difficult to study. The divergent functional and evolutionary requirements imposed on the centromere in various model organisms make such a study even more challenging. I propose a study of centromere architecture from a purely functional and mitosis-centric view-point. During mitosis, the main function of the centromere is to assemble a kinetochore with precisely defined protein architecture, so that the kinetochore can bind persistently to the plus-ends of a set number of microtubules (MTs). Moreover, the end-on geometry of the kinetochore-MT attachment, along with kinetochore proteins, is highly conserved (constrained by the MT lattice). It is likely necessary for proper kinetochore-mediated spindle assembly checkpoint function. Protein architecture of the kinetochore-MT attachment, defined as the spatial arrangement of kinetochore proteins with respect to the MT plus-end, thus provides a stringent test on the architecture of the kinetochore-chromatin interface. I am developing a strategy for deducing the *in vivo* protein architecture of the kinetochore-MT attachment using fluorescence microscopy. This strategy uses quantification of sensitized emission due to Forster Resonance Energy Transfer (FRET) along with structural and copy number data to deduce the distribution of kinetochore proteins with respect to the MT plus-end in budding and fission yeast. Preliminary data show that (1) Ndc80 complex molecules at least dimerize *in vivo*, (2) this is facilitated by the 139 amino acid unstructured domain at the N-terminus of Ndc80, (3) there is little stagger along the MT axis between adjacent Ndc80 molecules, and (4) this configuration of the Ndc80 complex gets more compact in anaphase. This new view of the kinetochore architecture provides key insight into the molecular mechanism of force generation. It also provides a road-map for systematically exploring architecture of the protein interface between the MT-binding and chromatin-binding domains of the kinetochore. In the long term, this strategy will specify the configuration of the centromere-kinetochore interface that the centromere must guarantee for successful chromosome segregation during mitosis.

Plasticity and Epigenetic Inheritance of CENP-A Nucleosome Positioning in the Fission Yeast Centromere. Jianhui Yao[1], Xingkun Liu[1], Takeshi Sakuno[2], Shawn P. Balk[1], Yuanxin Xi[3], Wei Li[3], Yoshinori Watanabe[2] and Xiangwei He[1]. [1]Department of Molecular and Human Genetics, Baylor College of Medicine [2]Laboratory of Chromosome Dynamics, Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi, Tokyo 113-0032, Japan. [3]Division of Biostatistics, Dan L.Duncan Cancer Center and Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

Nucleosomes containing the specific histone H3 isoform – Cenp-A, mark the centromere and initiate kinetochore assembly. For the common type of regional centromeres, little is known in molecular detail of centromeric chromatin organization, its propagation and how the distinct organization pattern may facilitate kinetochore assembly. Here, we show that in a genetically homogeneous cell population, the positions of Cnp1 nucleosomes within the centromeric core are highly flexible and variable among the cells. Consistently, a large portion of the endogenous centromere is dispensable for cell growth. We further show that variable Cnp1 occupancy directly correlates with stochastic silencing of the underlying reporter genes, and is largely inherited epigenetically throughout cell generations. Cnp1 nucleosomes also reposition frequently, the rate of which correlates directly with the length of the centromeric core and is enhanced by the histone chaperone Vps75. Together, our results reveal the plasticity in Cnp1 nucleosome positioning, and suggest a mechanism for its epigenetic inheritance.

The requirement for the Dam1 complex is dependent upon the number of kinetochore proteins and microtubules. Laura S. Burrack, Shelly E. Applen and Judith Berman. Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis MN USA

The Dam1 complex attaches the kinetochore to spindle microtubules and is a processivity factor *in vitro*. In *Saccharomyces cerevisiae*, which has point centromeres that attach to a single microtubule, deletion of any Dam1 complex member results in chromosome segregation failures and cell death. In *Schizosaccharomyces pombe*, which has regional centromeres that attach to 3-5 kinetochore microtubules per centromere, Dam1 complex homologs are not essential. To ask why the complex is essential in some organisms and not others, we used *Candida albicans*, a fungal species with regional centromeres that attach to a single microtubule. Interestingly, the Dam1 complex was essential in *C. albicans*, suggesting that the number of microtubules per centromere is critical for its requirement. Importantly, by increasing CENP-A expression levels, more kinetochore proteins and microtubules were recruited to the centromeres, which remained fully functional. Furthermore, Dam1 complex members became less essential for cell growth in cells with extra kinetochore proteins and microtubules. Thus, the requirement for the Dam1 complex is not due to the DNA-specific nature of point centromeres. Rather, the Dam1 complex is less critical when chromosomes have multiple kinetochore complexes and microtubules per centromere, implying that it functions as a processivity factor *in vivo* as well as *in vitro*.

Genome-wide identification of replication origins in *Candida albicans*. Meleah Hickman, Hung-Ji Tsai, Amnon Koren, Laura Burrack, and Judith Berman

DNA replication is an essential biological process and the necessary machinery is well conserved among eukaryotes. However, the genomic features that specify origins of replication (ORIs) and replication timing are not well understood. Current replication timing data supports a stochastic origin-firing model where the most efficient origins are in fact the earliest firing. We have found that centromeres are highly efficient, constitutive ORIs in *Candida albicans*. These centromeric origins not only bind the origin replication complex (ORC) with high affinity; they also have asymmetric GC skew patterns. These skew patterns are well conserved at centromeres in other *Candida* species as well as the distantly related *Yarrowia lipolytica* and indicate that the centromeric origins are extremely old. Non-centromeric ORIs are difficult to identify because there are many more ORC binding sites than active origins, and most ORIs do not appear to be constitutive. We are using comparative genomics with the yeasts *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Schizosaccharomyces pombe* to identify features associated with replication origin activity in order to characterize active, non-centromeric ORIs in *C. albicans*. Alignment of ORC binding sites with genome-wide nucleosome position revealed a strong correlation between ORC binding and nucleosome-depletion. Furthermore, a 50 bp motif recently identified in *K. lactis* as an ARS (autonomously replicating sequence) consensus sequence (ACS) appears in a subset of ORC binding sites in *C. albicans*. Currently, our results suggest that active replication origins in *C. albicans* fire in an evolutionarily conserved manner based on sequence features and chromatin-dependent factors.

Comparative functional genomics of two *Saccharomyces* yeasts. Maitreya Dunham. University of Washington, Seattle, WA

Although many fungal strains and species have been sequenced, experimental annotation of these genomes has not kept pace. However, functional studies in these genetically diverse isolates could be very informative in understanding their evolution and ecology. We have chosen one of these understudied species, *Saccharomyces bayanus*, in which to investigate these topics. Using a data-driven approach informed by the deep *S. cerevisiae* literature, we collected over 300 gene expression arrays for conditions found to be highly informative in the sister species. Comparison of the gene expression networks between the two species paints a complex picture of conservation and divergence over 20 million years. Further expression analysis in interspecific hybrids has helped determine which of these changes are determined in *cis* and *trans*. We have paired this analysis with additional comparative studies between the species, including ortholog knockout phenotypes and essentiality, DNA replication dynamics, nucleosome profiling, and behavior over experimental evolution timecourses. In all cases, key components show interesting changes, ranging from differences in replication timing of entire chromosome domains to subtle changes in affinity of nutrient transporters. Integration of these datasets with comparative sequence analysis promises to capture a high resolution picture of species-level evolution. We also hope that our methods will be informative for studies in other sequenced but otherwise understudied species.

DOI: 10.4148/1941-4765.1066

Concurrent Sessions II

Fungus-Host Signaling (Howlett/Döhlemann) **Merrill Hall**

Developmental and metabolic switches controlling infection in the trans-kingdom pathogen *Fusarium oxysporum*. Antonio Di Pietro, David Turra, Manuel S. Lopez-Berges, David Segorbe, Elena Perez-Nadales. Departamento de Genetica, Universidad de Cordoba, 14071 Cordoba, Spain. ge2dipia@uco.es

In the presence of the host, fungal pathogens undergo a developmental and metabolic switch towards infectious growth. Activation of the infection program leads to directed growth towards the host, penetration of the surface, invasion of underlying tissues and induction of disease symptoms. The soilborne fungus *Fusarium oxysporum* causes vascular wilt disease on over a hundred different plant species and superficial to invasive infections in immunocompromised humans. We are interested in the environmental and host-derived stimuli and cellular pathways that regulate infectious growth of *F. oxysporum* on plant or mammalian hosts. One of the key players in plant pathogenicity is Fmk1, a conserved mitogen-activated protein kinase (MAPK) Fmk1 that controls infection-related processes such as chemotropism, adhesion, root penetration and invasive growth. Most of these functions also require the downstream homeodomain transcription factor Ste12 and Msb2, a mucin-type membrane protein functioning in MAPK activation. The same processes are repressed in the presence of the preferred nitrogen source ammonium through a mechanism that requires the ammonium transporter MepB and the bZIP factor MeaB, and can be reversed by rapamycin, an inhibitor of TOR. These results suggest a possible link between nutrient regulation and MAPK signalling in the control of plant infection. Strikingly, the Fmk1 cascade is dispensable for virulence of *F. oxysporum* on immunodepressed mice, while other developmental and metabolic regulators such as Velvet, PacC and HapX contribute to infection of mammalian hosts.

Decoding symbiosis - molecular insights into the basis of grass-fungal interactions. Carla Eaton[1,*], Murray Cox[1], Chris Schardl[2], Barry Scott[1] [1]Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand; [2]Department of Plant Pathology, University of Kentucky, Lexington, Kentucky, USA [*]New address: Department of Plant Pathology and Microbiology, University of California Riverside, USA

Interactions, or symbioses, between plants and fungi span a broad continuum from mutualism to pathogenism. To gain insight into the molecular basis of plant-fungal symbiosis we utilised a mutant of the mutualistic grass endophyte *Epichloë festucae* which displays a switch from mutualistic to pathogenic association with perennial ryegrass. Using high throughout mRNA sequencing we identified fungal and plant genes that were differentially expressed between the mutant, pathogenic interaction and a wild-type association. Fungal gene expression changes were consistent with a switch towards unrestricted, pathogenic growth. These included up-regulation of hydrolases and transporters, which likely facilitates the striking increase in biomass *in planta* displayed by this mutant. There was also a dramatic metabolic shut-down of gene clusters involved in the production of host bioprotective molecules. Changes in host gene expression reflected the stressed state induced by infection with the mutant fungus, including activation of host-defense related gene expression and activation of transposases. Changes in expression of genes involved in the biosynthesis or response to nearly all major classes of plant hormones were also detected. These changes were consistent with phenotypic changes exhibited by the host, including stunted growth and precocious senescence. Using this approach we identified genes that are required for maintenance of mutualism or prevention of pathogenism between *E. festucae* and perennial ryegrass. This study highlights the power of high throughput mRNA sequencing for investigating plant-fungal interactions.

Interactions Between Dothideomycete Necrotrophic Effectors and Receptors in Wheat. Timothy L. Friesen, Zhaohui Liu, Shunwen Lu, Peter S. Solomon, Richard P. Oliver, and Justin D. Faris. USDA-ARS Northern Crop Science Lab, Cereal Crops Research Unit, Fargo ND 58102. Email: timothy.friesen@ars.usda.gov

Several of the Dothideomycete pathogens produce necrotrophic effectors (host selective toxins) that elicit susceptibility. Recent research indicates that recognition of these effectors is governed by host genes with resistance gene-like signatures including both nucleotide binding (NB) and leucine rich repeat (LRR) domains that act to confer susceptibility rather than resistance to the necrotrophic pathogens. It would appear that in several cases, necrotrophic pathogens use a mechanism whereby effectors are secreted into the host environment to elicit programmed cell death (PCD) followed by pathogen proliferation and sporulation. This is in contrast to classical effector triggered immunity (ETI) where effector recognition followed by R gene signaling results in resistance. The *Stagonospora nodorum*-wheat interaction is a classic example of a necrotrophic interaction. Multiple effector-host gene interactions have been identified and studied in this system. Recently we showed that *Tsn1*, the wheat gene that mediates recognition of the necrotrophic effector SnToxA, harbors resistance gene-like NB and LRR domains, and that many of the effector-host gene interactions show hallmarks of programmed cell death including up regulation of defense response genes, induction of reactive oxygen species (ROS) and DNA laddering but with the end result being susceptibility rather than resistance.

Manipulation of plant defense signaling by *Ustilago maydis* effectors. Gunther Doehlemann, Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Str. 10, D-35043 Marburg, Germany
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The smut fungus *Ustilago maydis* infects primordia of all aerial organs of its host plant maize. A biotrophic interaction is established immediately upon host penetration and is maintained during the entire interaction up to the formation of sexual spores within the tumor tissue. During disease progression, development of the infected plant tissue is reprogrammed to feed the proliferating fungal cells and form tumors. This fungal lifestyle essentially requires efficient suppression of plant defense responses, in particular host cell death. Small proteins secreted by thy fungus, so called effectors, are considered to be instrumental for the suppression of plant defense. While analysis of various *U. maydis* effector mutants confirmed important roles of these secreted proteins for virulence, only little is known about their actual molecular function. Therefore, our central aim is to understand how *U. maydis* effectors interact with particular host cellular processes and which plant signaling cascades are manipulated to trigger susceptibility.
Our recent findings suggest that suppression of maize cysteine proteases is a key process for the establishment of compatibility to *U. maydis*. This involves both the activity of fungal effectors as well as a specific regulation of host factors via hormone signaling pathways. In the presentation, our current knowledge on this regulatory network will be discussed.

The impact of the mycorrhizal symbiosis on the transcriptome of *Laccaria bicolor* and Poplar. Annegret Kohler[1], Jonathan M. Plett[1], Emilie Tisserant[1], Minna Kemppainen[2], Valérie Legué[1], Claire Veneault-Fourrey[1], Annick Brun[1], Alejandro G. Pardo[2]and Francis Martin[1]
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Mycorrhizal symbioses are universal in terrestrial ecosystems and may have been fundamental to land colonization by plants. Boreal and temperate forests all depend on ectomycorrhizae. These fungi are not an evolutionarily distinct group, but rather evolved several times from saprotrophic ancestors. Identification of the primary factors that regulate symbiotic development and metabolic activity will therefore open the door to understand the role of ectomycorrhizae in plant development and physiology, allowing the full ecological significance of this symbiosis to be explored. With the genome sequences of *Populus trichocarpa* and *Laccaria bicolor* in hand, we were able to investigate the transcriptome of both partners during mycorrhizal development. By using Nimblegen whole genome expression arrays and RNA-Seq we identified candidate genes from the fungus and its host tree. Amongst the most highly symbiosis up- regulated transcripts in *Laccaria bicolor* were several small secreted proteins. From amidst these small secreted proteins we have demonstrated that *MYCORRHIZAL iNDUCED SMALL SECRETED PROTEIN7* is an indispensable signal needed for the establishment of symbiosis. MiSSP7 is secreted by the fungus upon receipt of diffusible signals from plant roots, imported into the plant cell via endocytosis and targeted to the plant nucleus where it alters the transcriptomic fate of the plant cell. Further, *L. bicolor* transformants with severely reduced expression of MiSSP7 do not form functional mycorrhia with poplar roots. Martin, F. et al., The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature 452, 88-92 (2008)

***Aspergillus fumigatus* conidia modulate the endocytic pathway of alveolar macrophages.** Andreas Thywissen[1,3], Thorsten Heinekamp[1], Hans-Martin Dahse[2], Peter F. Zipfel[2, 3], and Axel A. Brakhage[1, 3]. [1] Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute (HKI), Department of Molecular and Applied Microbiology, Jena, Germany. [2] Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute (HKI), Department of Infection Biology, Jena, Germany. [3] Friedrich Schiller University, Jena, Germany.

The mould *Aspergillus fumigatus* is the main causative agent of invasive pulmonary aspergillosis in immunocompromised patients. Infection starts with the inhalation of *A. fumigatus* conidia that germinate in the lung. Professional phagocytes like alveolar macrophages contribute to the efficient clearance of fungi from the lung by phagocytosis and degradation of conidia followed by release of chemokines and cytokines in order to trigger neutrophil migration at the site of infection. In the immunocompromized host, at least some conidia are able to evade macrophage degradation, resulting in germination and outgrowth of intracellularly residing spores. Therefore, conidia must be able to evade recognition and processing by phagocytes. The avirulent *pksP* mutant of *A. fumigatus* lacking the melanin layer present on wild-type conidia exhibited increased phagocytosis by macrophages apparently due to the loss of masking immunogenic glucan-structures. Furthermore, by analysing phagolysosome fusion and acidification we show that intracellular processing of *pksP* mutant conidia is drastically increased in comparison to wild-type conidia, suggesting that *A. fumigatus* conidia interfere with the endocytosis pathway, similar to obligate human pathogens like *Legionella sp*. or *Mycobacterium sp*.. The process by which wild-type conidia mediate endocytotic alterations seems to be connected to the surface structure of melanized conidia but is independent of the presence of a functional RodA-derived rodlet layer. Moreover, inhibition of phagolysosome acidification by macrophages is controlled by the fungal cAMP signaling pathway.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Pathogen-caused release of linolenic acid suppresses plant defense by inhibition of callose synthesis in wheat. Voigt, Christian [1], Goebel, Cornelia [2], Bode, Rainer [2], Feussner, Ivo [2], and Schaefer, Wilhelm [1]. [1] Molecular Phytopathology, Biocenter Klein Flottbek, University of Hamburg, Germany. [2] Department of Plant Biochemistry, Georg August University, Goettingen, Germany.

The precise function of callose in papillae has not been shown unequivocally. We demonstrate that upon infection of wheat spikes with the fungal plant pathogen *Fusarium graminearum*, callose synthase activity and callose deposition are suppressed, and wheat is susceptible to fungal spreading. The secreted lipase FGL1 is an important virulence factor for *F. graminearum*. In contrast to *F. graminearum* wild-type, the lipase-deficient Î”fgl1 mutant is unable to suppress wheat callose synthesis. Wheat spikes are resistant to colonization by this mutant. Long- chain unsaturated free fatty acids (FFA) inhibit plant callose synthesis in vitro and in planta; and the previously observed resistance of the wheat spike to Î”fgl1 is broken. The lipase-deficient fungal mutant is able to colonize the spike. Analysis of the FFA level in wheat spikes during infection revealed an elevated linolenic acid concentration during *F. graminearum* wild-type compared to Î”fgl1 infection. We conclude that linolenic acid plays a decisive role in callose synthesis suppression during wheat â€“ *F. graminearum* interaction. A proposed model explains this novel mechanism of plant defense suppression by pathogen-caused increase in FFA due to lipase secretion.

Cellular pathways activated in the necrotrophic fungus Alternaria brassicicola in response to camalexin exposure. Thomas Guillemette, Aymeric Joubert, Claire Campion, Nelly Bataillé-Simoneau, Beatrice Iacomi-Vasilescu, Pascal Poupard, Philippe Simoneau. IFR QUASAV, UMR PaVé 77, 2 Bd Lavoisier, F 49045 Angers, France

Camalexin, the characteristic phytoalexin of Arabidopsis thaliana, inhibits growth of the fungal necrotroph Alternaria brassicicola. This plant metabolite probably exerts its antifungal toxicity by causing cell membrane damage. Here we observed that activation of a cellular response to this damage requires the unfolded protein response (UPR) and two MAPK signalling pathways, the cell wall integrity (CWI) and the high osmolarity glycerol (HOG). Camalexin was found to activate both AbHog1 and AbSlt2 MAP kinases in a precocious manner, and activation of the latter was abrogated in an AbHog1 deficient strain. Mutant strains lacking functional MAP kinases or AbHacA, the major UPR transcription regulator, showed in vitro hypersensitivity to camalexin and brassinin, a structurally related phytoalexin produced by several cultivated Brassica species. Enhanced susceptibility to the membrane permeabilization activity of camalexin was also observed for deficient mutants. These results suggest that the three signalling pathways have a pivotal role in regulating a cellular compensatory response to preserve cell integrity during exposure to camalexin. Replacement mutants exhibited a loss or an attenuation of the virulence on host plants that may partially result from their inability to cope with defence metabolites such as indolic phytoalexins. This constitutes the first evidence that a phytoalexin activates fungal MAP kinases and UPR, and that outputs of activated pathways contribute to protecting the fungus against antimicrobial plant metabolites. A functional model of fungal signalling pathways regulated by camalexin is proposed and leads to consider new promising strategies for disease control.

Interactions between Fungi and Prokaryotes (Pawlowska/Bonfante) **Kiln**

Understanding chemical crosstalk between bacteria and fungi in biofilms. Diana K. Morales, Nicholas J. Jacobs, Sathish Rajamani, Malathy Krishnamurthy, Juan R. Cubillos-Ruiz, Carla Cugini and Deborah Hogan. Dartmouth Medical School, Vail 208, Hanover, NH 03755

In addition to modifying the collective behavior of fungal and bacterial single-species populations, extracellular signaling molecules can shape interspecies interactions occurring within polymicrobial communities. *Pseudomonas aeruginosa* and *Candida albicans*, two microorganisms frequently found together in the clinical setting, have been found to undergo multiple interactions mediated by secreted microbial molecules. When these two microorganisms form mixed-species biofilms, *P. aeruginosa* induces fungal killing by secreting toxic phenazines. As a response, *C. albicans* senses extracellular bacterial molecules and changes its morphology to promote fungal dispersal. During this process *C. albicans* secretes farnesol, a molecule that is then sensed by *P. aeruginosa* and that stimulates the synthesis and secretion of phenazines. Interestingly, many of these bacterial-fungal interactions are most apparent when the organisms are grown in surface-associated biofilm communities, where unstable and poorly soluble molecules can exert their effects more efficiently. Studying *P. aeruginosa* and *C. albicans* interactions has unveiled novel chemical crosstalk strategies, which may play a crucial role in pathogenesis and microbial ecology. Hence, a complete understanding of the molecular mechanisms underlying these processes could reveal new approaches to confront mixed-species infections. Diana.K.Morales.Gamba@Dartmouth.edu

Interaction between Streptomycetes and *Aspergillus nidulans*. Hans-Wilhelm Nützmann[1 & 5], Volker Schroeckh[1], Kirstin Scherlach[2], Wolfgang Schmidt-Heck[3], Karin Martin[4], Christian Hertweck[2 & 5], and Axel A. Brakhage[1 & 5]; 1 Department of Molecular and Applied Microbiology, 2 Department of Biomolecular Chemistry, 3 Systems Biology/Bioinformatics Group, 4 Bio Pilot Plant Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute (HKI) and & 5 Friedrich Schiller University Jena. HKI Jena, Beutenbergstrasse 11a, 07745 Jena, Germany hans-wilhelm.nuetzmann@hki-jena.de

Microorganisms as bacteria and fungi produce many important low-molecular weight molecules that show different biological activities. Genome mining of fungal genomes indicated that their potential to produce these compounds designated secondary metabolites is greatly underestimated. However, most of the fungal secondary metabolism gene clusters are silent under laboratory conditions. Therefore, a major challenge in this emerging area is to understand the physiological conditions under which these compounds are produced. Results in this area will lead to the discovery of new bioactive compounds and to new insights in fundamental aspects of communication between microorganisms. To address these questions the important model fungus Aspergillus nidulans was coincubated with 58 different Streptomycetes. With one particular species, a specific interaction was shown. For the first time, using microarray analyses at the molecular level it was demonstrated that this interaction leads to the specific activation of two distinct silent secondary metabolism gene clusters. Electron microscopy confirmed the intimate interaction of the fungus and the bacterium. Full genome arrays of A. nidulans were applied to elucidate the whole genome response to the streptomycete. Data on the molecular regulation of the involved secondary metabolism gene clusters will be presented.

Unraveling the biological activities of a bacterial metabolite using *Saccharomyces cerevisiae* and *Neurospora crassa* as model organisms. Danielle Troppens, Olive Gleeson, Lucy Holcombe, Fergal O'Gara, Nick Read[1] and John Morrissey. Microbiology Department, University College Cork, Ireland, [1]Institute of Cell and Molecular Biology, University of Edinburgh, UK

Secondary metabolites are a rich source of antimicrobial and other bioactive molecules, mainly due to the frequent capacity to affect metabolism and other cellular processes in non-producing organisms. We are interested in the secondary metabolite 2,4-diacetylphloroglucinol (DAPG), produced by some *Pseudomonas fluorescens* strains. It exhibits a broad spectrum of antimicrobial activity but little is known about its cellular targets or possible fungal resistance mechanisms. We are using two model organisms, *S. cerevisiae* and *N. crassa*, to address these questions. DAPG treatment impairs cell growth in both organisms and causes loss of mitochondrial membrane potential suggesting that electron transport is a target. A genome-wide screen revealed that alterations of several processes, such as protein biosynthesis and DNA repair, can confer resistance. We also found that in both *S. cerevisiae* and *N. crassa*, DAPG induces a transient cytoplasmic Ca2+ signal. Using an aequeorin reporter system to monitor the Ca2+ signal we show that it originates in the external medium but is not transported exclusively via known channels. In addition to providing information on the antifungal mode of action of DAPG, this work may have broader significance in understanding interactions between bacterial and eukaryotic cells.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Ants, agriculture, and antibiotics. Cameron R. Currie and Jonathan L. Klassen. Department of Bacteriology, University of Wisconsin-Madison, Madison, WI

Fungus-growing ants cultivate specialized fungi for food. In exchange, the ants provide their cultivar fungus with substrate for growth, dispersal to new colonies, and protection from competitors. This ancient and obligate ant-fungus mutualism is known to occur alongside at least one other mutualist and two specific pathogens. Specifically, a specialized, coevolved fungal pathogen in the genus *Escovopsis* parasitizes ant fungus gardens, thereby destroying the ant's food source. To help overcome this garden pathogen, the ants have formed a mutualistic association with an antibiotic-producing actinobacterium that suppresses the growth of *Escovopsis*. This actinobacterium may in turn be parasitized by a black yeast, thereby lessoning its protective capacity versus *Escovopsis*. Inhibitory interactions between different members of this symbiosis vary significantly throughout its phylogenetic breadth, implying dynamic evolution of both antimicrobial interactions and concomitant resistance. How antibiotic diversification occurs within the context of a predominantly (but not exclusively) vertically-inherited symbiosis remains unknown. We have sequenced the genomes of several ant-symbiont actinobacteria and several of their closest free-living relatives to better understand both the extent to which symbiosis has uniquely shaped their genomes and also the extent of biosynthetic diversity that they encode. Our results reveal many strain-specific secondary metabolite clusters, even within symbiosis-specific phylogenetic lineages, and highlight the potential of these strains to produce novel pharmaceuticals.

Lichen symbioses as microecosystems. Martin Grube[1], Gabriele Berg[2]. [1]Institute of Plant Sciences, Karl-Franzens-University, Holteigasse 6, A-8010 Graz, Austria. [2]Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12, A-8010 Graz, Austria

Microbial consortia of bacteria and fungi play an important role in natural ecosystems. New molecular and microscopic techniques revealed progress in the understanding of fungal-bacterial symbioses. Lichens are traditionally considered as a self-sustaining association of fungi and photoautotrophic species. Our research during the last years revealed lichens as micro-ecosystems, which harbour highly abundant and diverse bacterial communities [1]. The analyses of samples from different lichen species by a polyphasic approach showed biofilm-like structures and a species specificity of the bacterial communitites [2]. Lichen-associated microbial communities consist of diverse taxonomic groups. The majority of bacteria belong to *Alphaproteobacteria* [3] but there are also new phylogenetic lineages. We observed that young and actively growing thallus parts of lichens host communities that are different from those of old and decaying parts. Until now, the function and interaction within the microbial consortia is not fully understood. The functions displayed mainly by culturable strains suggest that bacteria have lytic activities, complement the nitrogen budget and produce bioactive substances, including hormones and antibiotics. Environmental proteomics of *Lobaria pulmonaria* showed more proteins of prokaryotes than of the green algal photobionts in lichens, and suggested yet unexplored functions in the consortium. Furthermore, lichen-associated bacterial communities are an interesting source for biotechnology [4].
[1] Grube & Berg; Fungal Biology Reviews 23:72–85. [2] Grube et al.; The ISME J., 3:1105-1115. [3] Cardinale et al.; FEMS Microb. Ecol. 66: 63– 71. [4] Gasser et al.; IOBC Bull., in press.

Ectomycorrhizal fungi and their bacterial associates: what's new about the mechanisms of their interactions? Aurélie Deveau[1], Peter Burlinson[1,3], Angela Cusano[1], Abdala Diedhiou[1], Stéphane Uroz[1], Alain Sarniguet[2], Gail Preston[3], Pascale Frey-Klett[1]. [1]UMR 1136 IAM, INRA Nancy, France; [2]UMR 1099 BIO3P, INRA Rennes, France; [3] Department of Plant Sciences, University of Oxford, UK

In natural environments mycorrhizal fungi are surrounded by and shape complex bacterial communities. These bacteria modulate the mycorrhizal symbioses between soil fungi and plant root systems, forming an intimate tripartite multitrophic association. From these communities, so-called "Mycorrhiza Helper Bacteria" (MHB) can be identified that either assist mycorrhiza formation or interact positively with the functioning of the symbiosis (Frey-Klett *et al*., 2007). A thorough understanding of how MHB directly or indirectly promote plant health, growth and nutrition will enhance reforestation efforts and improve crop yields. In conjunction with advances in genomics, the development of genetic and nucleic acid-based methods to dissect these interactions has yielded new insights into the mechanisms that control the ecology of these bacteria and their helper effect. An overview of our current knowledge of this particular functional group of bacteria will be presented, with particular attention paid to recent investigations into the role of bacterial metabolites and type III secretion in the interaction of one MHB, *Pseudomonas fluorescens* BBc6R8, with fungal hyphae. Future priorities for MHB research will be discussed with important relevance to the rapidly developing scientific field of fungal-bacterial interactions (Tarkka *et al*., 2009). klett@nancy.inra.fr Frey-Klett et al., 2007, New Phytol, 176: 22-36 Tarkka et al., 2009, Curr Genet, 55: 233-243

The genome of the obligate endobacterium of an AM fungus reveals an interphylum network of nutritional interactions. S. Ghignone°, A. Salvioli°, I.Anca°, E. Lumini°, S.Cruveiller§ G.Ortu°, P.Piffanelli*, L. Lanfranco° and P. Bonfante° ° Department of Plant Biology, University of Torino and IPP-CNR, Italy § CEA Institut de Génomique – Genoscope, Paris France * Parco tecnologico Padano, Lodi - Italy

Many AMF host endobacteria in their cytoplasm. To elucidate the role of the Gram negative endobacterium Candidatus Glomeribacter gigasporarum (CaGg), we sequenced its genome using a metagenomic approach which combined Sanger sequencing of fosmid clones from a Gigaspora margarita (the host) library with a 454 pyrosequencing of an enriched- endobacterial fraction. The final assembly led to 35 contigs, totalling 1.72 Mb, consisting of a chromosome and three plasmids. The genome features of CaCg place it in the Burkolderiaceae group, while metabolic networks analysis clustered CaCg with insect endobacteria, mirroring its obligate intracellular life-style. CaCg resembles an aerobic microbe, with no fermentative and limited energy-production capabilities via glycolysis and phosphate-pentose pathways. It depends upon its fungal host for C, P and N supply; its ability to synthesize amino acids is limited suggesting that most amino acids are imported from its host. In summary, the bacterial genome data of the first endosymbiont's endosymbiont revealed a novel context of intimate symbiosis between bacteria and fungi. Since the CaGg fungal host is itself an obligate biotroph dependent on its photosynthetic host, our work uncovers a network of nutritional/evolutionary interactions between plants, AM fungi and endobacteria.

400 Million Year Old Facultative Dependence of Arbuscular Mycorrhizal Fungi on Glomeribacter Endobacteria. Stephen J. Mondo[1], Kevin H. Toomer[1], Joseph B. Morton[2], and Teresa E. Pawlowska[1] [1]Department of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY 14853-5904; [2]Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506-6108

Evolutionary theory predicts that over long periods of time, reciprocal selection in mutualistic endosymbioses will lead to increased symbiont interdependence and strict vertical transmission of endosymbionts. We tested this prediction in the mutualistic symbiosis between arbuscular mycorrhizal fungi (Glomeromycota) and *Ca*. Glomeribacter gigasporarum endobacteria. We generated multilocus datasets for both symbionts to examine their populations for evidence of cospeciation and recombination. We surveyed 115 isolates from 34 experimental fungal populations representing the diversity of the Gigasporaceae family. We found that even within closely related fungal groups, endobacterial presence varied, indicating that endobacteria are not essential for the survival of their hosts. However, despite being facultatively associated, we detected significant evidence for cospeciation between symbionts. This global pattern of cospeciation is largely the result of several significant contributions from relatively few lineages and weaker or no contributions from most others. Host switching and recombination amongst endosymbionts are the factors responsible for the absence of cospeciation in non-cospeciating host-endosymbiont pairs. As cospeciation implies simultaneous speciation of host and symbiont, we used the host fossil record to infer that the association of AM fungi with Glomeribacter is at least 400 million years old. Unlike most essential, obligate endosymbioses that have rapidly evolved from facultative interactions, the association between Glomeromycota and Glomeribacter appears to be permanently locked in a facultative state despite its ancient origin.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Emergent Fungal Diseases (Taylor/Fisher) **Chapel**

Policy to prevent the transport of pathogens. Sarah J Gurr[1] and Matthew Fisher[2] . sarah.gurr@plants.ox.ac.uk. [1]Department of Plant Sciences, University of Oxford, OX1 3RB, UK. [2]Imperial College, School of Public Health, St Mary's Campus, London W2 1PG, UK

This introductory talk will allude briefly to emerging fungal diseases of note, but with mention only of maladies of humans, bats and frogs. Thenceforth, greater emphasis will be placed on microbes which pose a significant threat to global food security and, in particular, the rice blast fungus *Magnaporthe oryzae* , with brief comment on the oomycete *Phytophthora infestans*.
Policy operates on vastly different scales (for example, from laboratory protocol to globally-binding legislation). It must be evidence-based. However, policy "ideal" and "reality" lie far apart, not least because our evidence-base is incomplete. We, as scientists, must gather robust data, raise awareness of the need for research, assess and communicate risk and be involved in policy formulation – but being mindful that the drivers which motivate scientists differ from those that influence policy-makers.
But what is policy? Can we develop a unifying global policy to prevent transport of pathogens? The answer is a resounding "no"; there is too much variation between fungal pathogens in terms of their disparate hosts, varying host specificity, differing life-cycles and modes of nutrition, different rates of emergence of virulent isolates and varying modes of dispersal - by aerial spores, via vectors or by transport of diseased tissue or by host migration. However, we can do much to mitigate real and potential disease by better understanding dispersal, improving pathogen surveillance and epidemiological models, and either boosting host immunity or using antifungals that specifically ablate germination. Further, the onus lies with the fungal scientific community to ensure that policy-oriented organisations at the national and international level (for instance, the FAO and the OIE) are able to access state-of-the-art information on risk to ensure effective and accurate decision making.
Finally, the talk will reflect on our knowledge-base regarding control of rice blast disease - from the perspective of changing pathogen virulence, host specificity in the face of global climate change, deployment of disease resistant cultivars and effective use of fungicides. It will conclude with a series of recommendations applicable to the control of fungal disease more generally.

Global molecular surveillance provides a framework for understanding diversity within the *Fusarium graminearum* species complex. Todd J. Ward[1], Kerry O'Donnell[1], Diego Sampietro[2], Anne-Laure Boutigny[3],and Altus Viljoen[3] [1] USDA-ARS, [2] Universidad Nacional de Tucumán, [3] University of Stellenbosch

Members of the *Fusarium graminearum* species complex (FGSC) are responsible for diseases of a variety of cereal crops worldwide. These species also are a significant food-safety concern because they contaminate grain with trichothecene mycotoxins that inhibit eukaryotic protein synthesis and can modulate immune function. In order to establish a global picture of FGSC diversity, a recently developed multilocus genotyping assay was used to assess species and trichothecene chemotype diversity among a world-wide collection of more than 8,000 FGSC isolates. The results revealed: 1) substantial regional variation in species and trichothecene chemotype composition and diversity, 2) recent changes in diversity and composition due to transcontinental movement, and 3) evidence of species-specific differences in host preference. In addition, the global population structure of *F. graminearum* was analyzed using variable number tandem repeat markers. Significant population differentiation was observed within this mycotoxigenic cereal pathogen, and evidence of the recent transcontinental movement of populations followed by limited genetic exchange between resident and introduced populations was uncovered.

From the Andes to Ireland: Tracking the worldwide migrations, host shifts and reemergence of *Phytophthora infestans*, the Plant Destroyer. Jean Beagle Ristaino, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695 Jean_Ristaino@ncsu.edu (919 515-3267)

Potato late blight, caused by the oomycete pathogen *Phytophthora infestans* is considered the most important biotic constraint to potato production worldwide and is a major threat to food security. New strains of the pathogen have migrated from South America on multiple occasions to cause significant losses for US growers. In 2009, potato and tomato late blight epidemics were the worst in modern history in the US due to a "perfect storm" of widespread inoculum distribution and conducive weather. We have previously examined the global migrations and evolutionary history of *P. infestans* in modern potato crops and identified the source and strain that caused the 19th century Irish potato famine from archival materials. We used mitochondrial DNA sequences and identified the Ia mtDNA haplotype of *P. infestans* in 19th century epidemics from Europe, the US and Ireland. Multilocus sequence data from nuclear and mitochondrial loci support an Andean origin of *P. infestans* and also suggest that the source of inoculum for the potato famine epidemics in Ireland were from the Andean region. We are currently tracking the waves of migration of the pathogen that occurred in the late 19th and early 20th century into the US using historic samples and have identified the mid 20th century migration of the Ib mtDNA haplotype and host shifts of the pathogen to wild *Solanum* species on two continents.

Comparative genomics of *Batrachochytrium dendrobatidis* reveals recombination in a single globalised hypervirulent lineage. Rhys A. Farrer[1], Trenton W. J. Garner[2], Francios Balloux[1], Matthew C. Fisher[1]. [1] The Department of Infectious Disease Epidemiology, Imperial College London, Norfolk Place, London, UK, [2]The Institute of Zoology, Zoological Society of London, Regent's Park London, UK

Batrachochytrium dendrobatidis (*Bd*) is a globally distributed generalist pathogen known to be a major cause for declines in amphibians. *Bd* has likely to have been present in populations of amphibians throughout the 20th century, but has since spread to new populations and locations through transmission and trade. To understand the origins of *Bd* populations and how it has adapted, we resequenced the genomes of 20 globally distributed isolates and mapped the distribution of polymorphism between isolates. We show that the majority of isolates, and all those that are associated with mass mortalities, comprise a single >99.9% genetically identical lineage with a pan-global distribution. However, we also discovered two separate, highly divergent lineages of *Bd* with different morphological and phenotypic characters. The Global Lineage of *Bd* manifests extensive inter-genomic phylogenetic conflict and genomic blocks showing loss-of-heterozygosity suggesting recent recombination in this clade. We hypothesise that ancestral recombination followed by anthropogenically-mediated global spread of the progeny has led to the disease-driven losses in amphibian biodiversity.

The emergence of *Geomcyes destructans* and bat white-nose syndrome in North America. David S. Blehert. US Geological Survey – National Wildlife Health Center, Madison, Wisconsin

White-nose syndrome (WNS) is a disease associated with unprecedented bat mortalities in the eastern United States and Canada. Since the winter of 2006-2007, bat population declines approaching 100% have been documented at some surveyed hibernacula. Total estimated losses have exceeded one million bats over the past three years. Affected hibernating bats often present with visually striking white fungal growth on their muzzles, ears, and/or wing membranes. Histopathological and microbiological analyses demonstrated that WNS is characterized by a hallmark fungal skin infection caused by a recently discovered species of psychrophilic (cold-loving) fungus, *Geomyces destructans*. The fungus was initially discovered by laboratory culture at 3˚C and grows optimally between 5˚C and 14˚C, temperatures consistent with the body temperatures of hibernating cave bat species from temperate regions of North America. Laboratory infection trials indicated that *G. destructans* is transmissible bat-to-bat, and DNA from the fungus has been identified in environmental samples collected from several bat hibernation caves within WNS-infested states. There is a growing body of evidence supporting an association between WNS and cutaneous fungal infection by *G. destructans*, and this disease represents an unprecedented threat to bats of temperate regions of North America and beyond. Worldwide, bats play critical ecological roles in insect control, plant pollination, and seed dispersal, and the decline of North American bat populations may have far-reaching ecological consequences.

Comparative genomics of human fungal pathogens causing paracoccidioidomycosis. Christopher Desjardins, Jason Holder, Jonathan Goldberg, Sarah Young, Qiandong Zeng, Brian Haas, Bruce Birren, Christina Cuomo, and the Paracoccidioides Genome Consortium The Broad Institute, Cambridge, MA 02141 cdesjard@broadinstitute.org

Paracoccidioides brasiliensis is a dimorphic fungal pathogen and the causative agent of paracoccidioidomycosis, a human systemic mycosis endemic to Latin America. In order to better understand the biology of *Paracoccidioides* we sequenced the genomes of three strains: Pb01, Pb03, and Pb18. We also placed the Pb18 assembly on an optical map consisting of 5 chromosomes. Compared to their non-dimorphic relatives, *Paracoccidioides* and other dimorphic fungi encode a reduced repetoire of genes involved in carbohydrate and protein metabolism and synthesis of secondary metabolites. To compare genome content with phenotypic ability to utilize substrates for growth, we tested the related non-pathogenic *Uncinocarpus reesii* in metabolic assays. *U. reesii* displays broader and more effective growth on proteins than carbohydrates, which may predispose the dimorphic fungi to a pathogenic lifestyle. Furthermore, *Paracoccidioides* and other pathogenic dimorphs show expansions of the fungal-specific kinase family FunK1 and rapid evolution of transcription factors, suggesting specialized signaling and regulation potentially involved in dimorphism. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No.: HHSN2722009000018C.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Population structure and virulence of *Cryptococcus gattii* from AIDS patients in southern California. Wenjun Li[1], Edmond Byrnes III[1], Ping Ren[2], Yonathan Lewit[1], Kerstin Voelz[3], Robin May[3], Sudha Chaturvedi[2], Vishnu Chaturvedi[2], Joseph Heitman[1]. [1] Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC. [2]Wadsworth Center, Albany, NY. [3]Department of Molecular Pathobiology, University of Birmingham, Birmingham, United Kingdom

C. gattii infections in Southern California have been reported to occur in immunocompromised patients, specifically patients with AIDS. In this study, we examined the molecular epidemiology, population structure, and virulence attributes of *C. gattii* isolates collected from a cohort of AIDS patients in Los Angeles County, California. We show that these isolates consist almost exclusively of VGIII molecular type, in contrast to the vast majority of VGII molecular type isolates found in the outbreak region of the North American Pacific Northwest. Upon molecular phylogenetic analysis, the global VGIII population structure can be divided into two groups, VGIIIa and VGIIIb. We show that isolates from the CA patients are virulent in murine and macrophage models of infection, with VGIIIa significantly more virulent than VGIIIb. Several VGIII isolates are highly fertile and able to produce large numbers of spores that may serve as infectious propagules. Based on molecular analysis, the **a** and alpha VGIII MAT loci are largely syntenic with the known VGI and VGII *MAT* loci. Our studies indicate that *C. gattii* VGIII is endemic in Southern California, with other isolates originating from the neighboring regions of Mexico, and in some rarer cases from Oregon and Washington State. Given that greater than 1,000,000 cases of cryptococcal infection occur annually in the context of the global AIDS pandemic, our findings suggest a significant burden of *C. gattii* infection in AIDS patients may be unrecognized, with potential prognostic and therapeutic implications. These results signify the need to classify pathogenic Cryptococcus cases in the region and elsewhere and highlight possible host differences among the *C. gattii* molecular types, influencing infection of immunocompetent (VGI/VGII) vs. immunocompromised (VGIII/VGIV) hosts.

“Magic traits” drive the emergence of pathogens. Pierre Gladieux[a,b], Fabien Guérin[b], Tatiana Giraud[a], Valérie Caffier[b], Christophe Lemaire[b], Luciana Parisi[b], Frédérique Didelot[b], Bruno Le Cam[b]. [a]Univ. Paris Sud/ CNRS, UMR Ecologie Systématique Evolution, Orsay, France; [b]INRA/Univ. Angers, UMR PaVé, Beaucouzé, France. E-mail: pierre.gladieux@u-psud.fr

An important branch of evolutionary biology strives to understand how divergent selection for an ecologically important trait can foster the emergence of new species specialized on different niches. Such ecological speciation is usually difficult to achieve because recombination between different subsets of a population that are adapting to different environments counteracts selection for locally adapted gene combinations. Traits pleiotropically controlling adaptation to different environments and reproductive isolation are therefore the most favourable for ecological speciation, and are thus called “magic traits”. We used genetic markers and cross-inoculations to show that pathogenicity-related loci are responsible for both host adaptation and reproductive isolation in emerging populations of *Venturia inaequalis*, the fungus causing apple scab disease. Because the fungus mates within its host and because the pathogenicity-related loci prevent infection of the non-host trees, host adaptation pleiotropically maintains genetic differentiation and adaptive allelic combinations between sympatric populations specific to different apple varieties. Such “magic traits” are likely frequent in fungal pathogens, and likely drive the emergence of new diseases.

Regulation of Septation During Growth and Development (Yarden/Seiler) **Heather**

Dynamics of septum formation in *Ashbya gossypii.* Andreas Kaufmann, Philippe Laissue, Claudia Birrer, Hanspeter Helfer, Shanon Seger and Peter Philippsen. Biozentrum University of Basel, Switzerland

We started our work on septation in *A. gossypii* ten years ago when research on septation in other filamentous fungi was already much advanced. Therefore we focused on a few specific questions, all related to our overall goal to identify key steps in the evolution of *A. gossypii* from a most likely budding yeast-like ancestor. The unexpected finding of a very high level of gene order conservation between *A. gossypii* and *S. cerevisiae* was the prime reason for this focus. With respect to septation in *A. gossypii* we wanted to identify differences and similarities to septum formation and degradation in S.
cerevisiae. *A. gossypii* carries homologs for all *S. cerevisiae* proteins known to be involved in septum formation. Using life cell imaging to determine dynamic parameters, electron microscopy to visualize structural details, and deletion mutants to infer functions of domains from phenotypes we could address a series of questions concerning septation in *A. gossypii*: Which factors promote the formation of the actomyosin ring and its contraction? Are septa completely closed? Which kinetic parameters differ significantly in both systems? Are there mutants which destabilize septa? Which factors are involved in selecting hyphal sites for septation? Can one visualize targeted membrane additions? Are eisosomes involved in septation? Answers to these questions show key differences to septation in *S. cerevisiae*.

Dynamics of actin and actin binding proteins during septum formation in *Neurospora crassa*. Mouriño-Pérez, Rosa R., Olga A. Callejas-Negrete, Diego L. Delgado-Alvarez, Ramón O. Echauri-Espinosa. Departamento de Microbiología, CICESE. Ensenada, Mexico. rmourino@cicese.mx .

Filamentous actin plays essential roles in filamentous fungi, as in all other eukaryotes, in a wide variety of cellular processes including cell growth, intracellular motility, and cytokinesis. We visualized F-actin organization and dynamics in living *N. crassa* via confocal microscopy of growing hyphae expressing GFP fusions with homologues of the actin- binding proteins fimbrin (FIM) and tropomyosin (TPM-1), a subunit of the Arp2/3 complex (ARP-3), coronin (cor1) and a recently developed live cell F-actin marker, Lifeact. All GFP fusion proteins studied were also transiently localized at septa: Lifeact-GFP first appeared as a broad ring during early stages of contractile ring formation and later coalesced into a sharper ring, TPM-1-GFP was observed in maturing septa, and FIM-GFP/ARP3/COR1-GFP labeled cortical patches formed a double ring flanking the septa. Our observations suggest that each of the N. crassa F-actin-binding proteins analyzed associates with a different subset of F-actin structures, presumably reflecting distinct roles in F-actin organization and dynamics during all the stages of septation. Actin is present since early stages of septum formation, the contractile force of the actomyosin ring is related to the presence of tropomyosin and it seems that there is a need of plasma membrane remodeling regards the presence of endocytic patches labeled by fimbrin, coronin and Arp2/3 complex.

Regulation of Septins assembly by Rts1 during *Candida albicans*. morphogenesis. [1]David Caballero-Lima, [2]Alberto Gonzalez-Novo, [1]Pilar Gutierrez-Escribano, [1]Carmen Morillo-Pantoja, [2]Carlos R. Vazquez de Aldana and [1]Jaime Correa- Bordes. 1 Ciencias Biomedicas. Facultad de Ciencias, Universidad de Extremadura, Avda Elvas sn, Badajoz 06071, Spain. Phone: +34924289300 ext 86874, Fax: +34924289300, e-mail: jcorrea@unex.es 2 Inst. Microbiologia Bioqca. Dpto. Microbiologia y Genetica. CSIC/Universidad de Salamanca.

Inhibition of cells separation is characteristic of hyphal growth in *Candida albicans*. This inhibition is dependent on Sep7 phosphorylation by the hyphal-specific cyclin Hgc1, which regulate dynamic of the septin ring. Here, we show the role of Rts1, a regulatory subunit of PP2A phosphatase, in septin ring regulation in yeast and hyphal growth. In yeast, Rts1-Gfp translocates transiently from the nucleus to the bud neck after actomyosin ring contraction and is mainly located at the daughter side of the septin collar. In accordance with this, yeast cells lacking RTS1 fail to split septin ring properly during citokynesis. Whereas in wild-type cells the septin collar divides in two septin rings of similar diameter, rts1 mutant cells ring at the side of the daughter is significantly wider than the mother. This difference correlates with buds having a bigger size than the mothers. Moreover, disassembly of septins rings is compromised at the end of citokynesis and they persist for several cell cycles. During hyphal induction, rts1 mutant cells show a pseudohyphal-like growth. Interestingly, septin rings were misshapen and some longitudinal septins filaments were observed at the tip of the apical cell. In addition, aberrant septins structures could be seen at the cortex of yeast and hyphae. Furthermore, all septins were analyzed by SDS-PAGE, finding there is an increase in Sep7 phosphorylation levels. These results indicate that Rts1 is necessary for the normal assembly of septins structures in *Candida albicans*.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Genetic regulation of septation in the dimorphic basidiomycete *Ustilago maydis*. Christian Böhmer, Johannes Freitag, Björn Sandrock and Michael Bölker, FB Biologie, Universität Marburg, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany; email: boelker@staff.uni-marburg.de

During its lifecycle the dimorphic basidiomycete Ustilago maydis adopts two major lifestyles. Haploid cells grow vegetatively by budding, while dikaryotic cells display hyphal tip growth. We have studied the septation events that occur during both stages. Budding cells separate by consecutive formation of two distinct septa: first, a septum is laid down at the mother-bud neck and then a secondary septum is formed within the daughter cell. We have analyzed the contribution of Rho GTPases for septum initiation and followed the dynamics of septin proteins during septation. Cytokinesis and cell separation during budding involves several transitions of septin structures that are triggered by two protein kinases. Gin4 kinase is involved in direct phosphorylation of septin proteins, while the germline centre kinase Don3 is specifically required for the septin collar-to-ring transition of the secondary septum. During filamentous growth of dikaryotic cells retraction septa are formed at the distal end of the growing hypha. These septa delimit empty compartments from the cytoplasm-filled tip cells. We could show that formation of retraction septa depends on the presence of the Don1/Cdc42/Don3 signalling module and involves formin-dependent actin polymerization. Interestingly, during plant infection retraction septa are prerequisite for appressorium formation in longer filaments.

Septal plugging is a dynamic process which depends on the environmental conditions. Robert-Jan Bleichrodt[1], Arend F. van Peer[1], Brand Recter[1], Wally H. Muller[3], Teun Boekhout[4], Jun-Ichi Maruyama[2], Katsuhiko Kitamoto[2], Luis G. Lugones[1] and Han A. B. Wösten[1]. [1]Department of Microbiology, Utrecht University and Kluyver Centre for Genomics of Industrial Fermentations. [2]Department of Biotechnology, University of Tokyo. [3]Department of Cellular Architecture and Dynamics, Utrecht University. [4]CBS Fungal Biodiversity Centre, Utrecht

Hyphae of ascomycetes and basidiomycetes are compartmentalized by septa. Septa contain a central pore which allows for cytoplasmic streaming and translocation of organelles. Upon damage septa can be closed. In basidiomycetes, septa are plugged by electron dense material. Evidence indicates that the septal pore cap is involved in this plugging process. The core of the septal pore cap of *Schizophyllum commune* is comprised of the proteins SPC33 and SPC14. In ascomycetes, septa are closed by Woronin Bodies. The core of these peroxisome-like organelles consists of the HEX1 protein.
Recently, we showed that septa are not only plugged upon mechanical damage. Septa can also be closed during vegetative growth. The incidence is affected by the environmental conditions. Septa of *S. commune* tend to be open in the absence of glucose. In contrast, they close when exposed to high temperature, hypertonic conditions, or to an antibiotic. By changing the temperature conditions, it was shown that plugging was reversible. In *Aspergillus oryzae*, hypertonic or low pH stress tends to close septa. On the other hand, low and high temperature, hypotonic conditions and high pH open more septa. We have shown that Woronin Bodies are responsible for the plugging of septa during vegetative growth. In contrast, localization of AoSO at septa highly increases the incidence of septal opening. Septal closure by Woronin Bodies and AoSO localization were shown to be reversible. Our data imply that *S. commune* and *A. oryzae* can regulate their intercompartmental cytoplasmic continuity by reversible closure of their septa.

The vacuolar membrane protein PRO22 from *Sordaria macrospora* is involved in septum formation in early sexual structures. Sandra Bloemendal[1], Kathryn M Lord[2], Kathrin Bartho[3], Ines Teichert[1], Dirk A Wolters[3], Nick D Read[2] & Ulrich Kück[1] [1]Department of General and Molecular Botany, Ruhr-University Bochum, Universitaetsstr. 150, D-44780 Bochum, Germany, sandra.bloemendal@rub.de [2]Institute of Cell Biology, University of Edinburgh, Rutherford Building, King's Buildings, Edinburgh EH9 3JH, UK [3]Department of Analytical Chemistry, University of Bochum, Germany.

The transition from the vegetative to the sexual cycle in filamentous fungi requires a multicellular differentiation process. For the homothallic ascomycete *Sordaria macrospora*, several developmental mutants are described. One of these mutants, pro22, produces only defective protoperithecia and carries a point mutation in a gene encoding a protein which is highly conserved throughout eukaryotes. Extensive microscopic investigations revealed that pro22 displays a defect in ascogonial septum formation, indicating that PRO22 functions during the initiation of sexual development. Live-cell imaging showed that PRO22 is localized in the tubular vacuolar network of the peripheral colony region close to growing hyphal tips, and in ascogonia, but is absent from the large spherical vacuoles in the vegetative hyphae of the subperipheral region. Our aim is to extend the functional analysis of PRO22 by identifying interaction partners *in vitro* via yeast two-hybrid and *in vivo* via tandem-affinity purification.

Antagonistic interaction between the RSC chromatin-remodeling complex and the septation initiation network in the filamentous fungus *Aspergillus nidulans.* Hwan-Gyu Kim, Cui J. Tracy Zeng, Jung-Mi Kim, and Bo Liu. Department of Plant Biology, College of Biological Sciences, University of California, 1 Shields Avenue, Davis, CA 95616, USA

Cytokinesis/septation is activated by the conserved septation initiation network (SIN) in the filamentous fungus *Aspergillus nidulans*. MOBA binds to the SIN kinase SIDB and plays an essential role in cytokinesis and asexual sporulation/conidiation. We have isolated *smo* (*s*uppressor of *mo*bA) mutations that restored septation and conidiation upon down regulation of MOBA or other SIN components. Here we report that the *smoA* gene encodes a nuclear protein with homologs only found in filamentous ascomycetes. An affinity chromatographic attempt resulted in the isolation of SMOA and at least 11 other proteins identified by a mass spectrometry-assisted approach. Reciprocal affinity purifications verified the presence of these 12 proteins in a protein complex resembling the RSC chromatin-remodeling complex of the SWI/SNF family. Mutational analysis showed that the core RSC components of RSC6 and RSC8 were essential for growth and colony formation, but most accessory components were not. Null mutations of the *arpH* and other non-essential genes significantly slowed down colony growth without affecting conidiation. These mutations also suppressed the septation and conidiation defects caused by the down regulation of the *sidB*, *mobA*, and other SIN genes. Taken together, our results indicated that the RSC complex acted antagonistically against the SIN pathway to regulate septation in *A. nidulans*.

Developmental regulation of septum formation in *Aspergillus nidulans*. Haoyu Si, William R. Rittenour, and Steven D. Harris, Dept. of Plant Pathology and Center for Plant Science Innovation, University of Nebraska, Lincoln, USA 68588-0660.

In filamentous fungi such as *A. nidulans*, hyphae are partitioned into cells via the formation of cross-walls known as septa. The use of genetic and cell biological approaches has provided considerable insight into the mechanisms underlying hyphal septation. Essentially, nuclear signals function through the septation initiation network to control the formin-mediated assembly of a contractile actin ring at the septation site. During asexual development, *A. nidulans* forms airborne conidiophores that consist of a stalk that supports a vesicle, which in turn produces metulae, followed by a phialide and spores. Notable, during conidiation, *A. nidulans* undergoes a morphogenetic transition from an acropetal to a basipetal growth pattern.. Concomitantly, septa also change in appearance during conidiation. As part of our characterization of *A. nidulans* homologues of yeast bud site selection proteins, we have found that Bud4 functions during septation in both hyphae and conidiophores. Conversely, Axl2 only functions during the septation event that separates spores from the phialide. Furthermore, components the Cdc42/Rac1 GTPase modules (i.e., Cdc24, Rga1, Cla4), which have no obvious role in hyphal septation, also localize to the phialide-spore septation site. Our observations imply that septum formation is developmentally regulated during conidiation in *A. nidulans*, and we suggest that this regulation is critical for the transition to a basipetal growth pattern.

DOI: 10.4148/1941-4765.1066

Secretion, Endocytosis and Membrane Trafficking (Shaw/Penalva) **Fred Farr Forum**

Exocytosis and tip growth in *Neurospora crassa*. Meritxell Riquelme[1], Robert W. Roberson [2], Salomon Bartnicki-Garcia[1] and Michael Freitag[3]. [1] Center for Scientific Research and Higher Education of Ensenada CICESE, Baja California, México. [2] School of Life Sciences, Arizona State University, Tempe, Arizona, USA. [3] Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR, USA.

In fungal hyphae, apical growth is supported by the constitutive exocytosis of secretory vesicles, which through a "full fusion" process supply the normal complement of proteins and lipids to extend the plasma membrane and the precursors and enzymes for building the cell wall. Previously, we have shown that in *Neurospora crassa* vesicles containing cell-wall building enzymes are transported along the hyphae and accumulate temporarily in the Spitzenkörper in a stratified manner. The Vesicle Supply Center (VSC) model for fungal morphogenesis predicted that these vesicles are distributed from the Spitzenkörper outwards in all directions, generating a sharp gradient of exocytosis, with a maximum at the pole and vanishing gradually in the subapex. Prior to SNAREs recognition, secretory vesicles are presumably tethered to their target acceptor membrane in a process mediated by the exocyst. We endogenously tagged with GFP the exocyst components SEC-3, SEC-5, SEC-6, SEC-8, SEC-15, EXO-70 and EXO-84 in *N. crassa*. Some components accumulated surrounding the frontal part of the Spitzenkörper, whereas others were found in a delimited region of the apical plasma membrane, which correlates with the place of intensive exocytosis during polarized growth. A more detailed analysis by TIRFM revealed that the fluorescently labeled exocyst components followed a pulsatile exocytotic process, suggesting an orderly mechanism for exocytosis of the vesicles constituting the Spitzenkörper. Our results indicate that the region of exocyst-mediated vesicle fusion at the hyphal apical plasma membrane has the same extension than the exocytosis gradient predicted earlier by the VSC model.

Functional analysis of SPFH domain-containing proteins, Flotillin and Stomatin, in *Aspergillus nidulans*. Norio Takeshita, Reinhard Fischer. Karlsruhe Institute of Technology, Karlsruhe, Germany, norio.takeshita@kit.edu

Polarized growth of filamentous fungi depends on the microtubule and the actin cytoskeleton. Apical membrane- associated landmark proteins, so-called "cell end markers" link the two cytoskeletons. Our latest results indicate that apical sterol-rich membrane domains (SRDs) play important roles in polarized growth and localization of cell end markers. The roles and formation mechanism of SRDs remain almost unknown. To analyze the functional roles of SRDs, we are investigating the mechanism of SRD (or raft cluster) formation and maintenance. There are numerous studies on raft formation in different organisms. Flotillin/reggie proteins for instance were discovered in neurons and are known to form plasma membrane domains. The flotillin/reggie protein and a related microdomain scaffolding protein, stomatin, are conserved in filamentous fungi but have not yet been characterized. We have started the investigation of their functions by gene deletion and GFP-tagging. It was revealed that the flotillin/reggie protein FloA-GFP accumulated at hyphal tips. Deletion of *floA* caused a reduction of the growth rate and often irregular shaped hyphae. Moreover, the stomatin related protein StoA-GFP localized at young branch tips and at the subapical cortex in mature hyphal tips. Deletion strains of *stoA* also showed smaller colonies than wild-type and exhibited irregular hyphae and increased branching. The localization of SRDs, cell end markers, and actin etc. are being analyzed in the mutants.

Cryptococcal WASp homolog Wsp1 functions as an effector of Cdc42 and Rac1 to regulate intracellular trafficking and actin cytoskeleton. Gui Shen[1], J. Andrew Alspaugh[2] and Ping Wang[1,3,4],[1]Research Institute for Children, Children's Hospital, New Orleans, Louisiana USA; [2]Departments of Medicine and Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina USA; and Departments of [3]Pediatrics and [4]Microbiology, Immunology and Parasitology, Louisiana State University Health Sciences Center, New Orleans, Louisiana USA,gshen@chnola-research.org

Cryptococcus neoformans is an opportunistic human fungal pathogen that infects mainly immunocompromised patients, causing meningoencephalitis. It is thought to secrete virulence associated factors into its environment, which indicates proteins involved in intracellular transport may be ideal anti-fungal targets. We have recently characterized a novel endocytic protein, Cin1, and found that Cin1 interacts with Wsp1, a WASP homolog, and Cdc42, a Rho family GTPase. We found that Wsp1 also plays an important role in morphogenesis, intracellular transport, and virulence of the fungus. Additionally, we found that Wsp1 tagged with DsRed co-localizes with the GFP-actin and the GFP-Arp2, suggesting Wsp1 has a conserved role in activating the Arp2/3 protein complex. Both the basic and the GTPase binding domain of Wsp1 appear to play an auto-inhibitory role, similar to mammalian WASp proteins. Activation of Wsp1 by Cdc42 resulted in plasma membrane distribution, suggesting a role in exocytosis, and loss of Cdc42 function caused disappearance of actin cables in the wsp1 mutant, indicating that Wsp1 is an effector of Cdc42. We also provided evidence demonstrating that Wsp1 is an effector of another Rho GTPase, Rac1, in the regulation of vacuolar morphology. Our combined data showed that functions of Wsp1 in intracellular trafficking, vacuole morphogenesis, and actin cytoskeleton are mediated through its role as an effector of both Cdc42 and Rac1. The knowledge gained may extend the current understanding of WASp and Rho-family small GTPases in other eukaryotic organisms.

Imaging actin dynamics in *Aspergillus nidulans* using Lifeact. Brian D. Shaw, Laura Quintanilla, Srijana Upadhyay, and Zaida Hager Dept. Plant Pathology and Microbiology, Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX 77843-2132, USA

Polarization of actin to the hyphal apex is essential for hyphal growth. Previous work in *A. nidulans* has shown a sub-apical collar of actin::GFP patches that is associated with endocytosis and is necessary for growth. Here we use the Lifeact construct, an actin binding domain fused to either GFP or RFP, to image both actin patches and cables during growth. In addition to the sub-apical collar of patches in growing cells, we also note actin cables organized to the Spitzenkorper in growing tips. We also report here a new structure that we term the Sub-apical Actin Web (SAW). The SAW can be described as a complex array of actin cables distal to the tip in growing cells. This array is stable on the distal face but is highly dynamic on the proximal face with cables bending, retracting and growing toward the apex. Results of co-localization studies will be discussed.

Motor cooperation in long-range motility of early endosomes. Martin Schuster, Shreedhar Kilaru, Gero Fink and Gero Steinberg. University of Exeter, Biosciences, EX4 4QD, Exeter, UK G.Steinberg@exeter.ac.uk

The intrinsic polarity of microtubules determines the organelle transport direction, with kinesins moving their cargo to plus-ends and dynein taking organelles towards minus-ends. In the fungus *Ustilago maydis* the plus-ends of the microtubules are located near the growing hyphal tip, whereas the minus-ends are concentrated in sub-apical regions of the cell. Microtubules serve as “tracks” for long-range motility of early endosomes that can reach up to 90 micrometers in a single run. Anterograde (tip-directed) motility of these organelles is supported by kinesin3, whereas dynein moves the early endosomes towards the cell center (retrograde). However, this concept is an oversimplification, as it does not consider the orientation of the underlying microtubule array. In fact, long-range motility of early endosomes requires unexpected cooperation of kinesin-3 and dynein.

Function of the microtubule-binding domain of dynactin p150 in microtubule-plus-end accumulation of dynein in *Aspergillus nidulans*. Xuanli Yao, Jun Zhang, Henry Zhou, Eric Wang and Xin Xiang. Department of Biochemistry and Molecular Biology, the Uniformed Services University, Bethesda, MD, USA

In filamentous hyphae, dynein accumulates at the plus ends of microtubules in a dynactin- and kinesin-1-dependent manner, and the plus-end accumulation of dynein is important for the minus-end-directed transport of early endosomes. We asked in *Aspergillus nidulans* whether the microtubule (MT)-binding region of the dynactin p150 subunit is required for this accumulation. The MT-binding region locates at the N-terminus of p150, and it includes a CAP-Gly (cytoskeleton-associated protein glycine-rich) domain and a basic domain rich in basic amino acids. Here we show that deletion of the MT-binding region causes a significant reduction of microtubule plus-end accumulation of both dynein heavy chain and p150, although it does not affect dynein-dynactin interaction. Surprisingly, deleting the CAP-Gly domain alone causes no apparent effect on the plus-end accumulation of p150 while deleting the basic domain significantly diminishes the plus-end accumulation of p150. In addition, loss of the basic domain results in a partial defect in nuclear distribution similar to that observed in the mutant without the MT-binding region, and also an abnormal accumulation of early endosomes at the hyphal tip. Our results demonstrate the in vivo importance of the basic domain of p150, and also suggest that p150 is able to accumulate at the microtubule plus-ends by using a mechanism(s) requiring no direct interactions between its CAP-Gly domain and other protein motifs.

Vacuole homeostasis by a balance of membrane fission and fusion. Lydie Michaillat, Tonie Baars and Andreas Mayer. Department of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland

Many organelles exist in an equilibrium between fragmentation and of fusion which determines their size, copy number and shape. Yeast vacuoles rapidly (<1 min) fragment into up to 15 smaller vesicles under hypertonic stress and they coalesce into one big organelle upon nutrient limitation or hypotonic stress. Vacuoles also fragment and fuse in response to the cell cycle and nutrient availability. We have screened mutants defective in vacuole fragmentation and we have reconstituted the fragmentation reaction in vitro with isolated organelles. The in vitro reaction faithfully reproduces in vivo vacuole fragmentation. By a combination of in vivo and in vitro approaches we show that surprisingly vacuole fragmentation (membrane fission) depends on several components of the membrane fusion machinery, e.g. specific SNAREs. In addition, we show that the TOR signaling pathway, which is regulated by upon starvation, induces coalescence of the vacuoles into one big organelle under these conditions. We discovered that TOR positively regulates vacuole fragmentation whereas it has no influence on vacuole fusion. The resulting selective downregulation of fragmentation explains the decrease of vacuole number and the increase of their size under starvation conditions. Our combined in vivo and in vitro approaches have the potential to elucidate the regulatory interplay of membrane fusion and membrane fission machinery that determine organelle structure.

The interface between the Golgi and the endosomal system in *Aspergillus nidulans* Miguel A. Peñalva and Areti Pantazopoulou. Department of Molecular and Cellular Medicine. CSIC Centro de Investigaciones Biológicas. Ramiro de Maeztu 9, Madrid 28040, Spain penalva@cib.csic.es

The *A. nidulans* early- and late-Golgi is formed by a dynamic network of rings, tubules and fenestrated structures that is strongly polarised. Early and late Golgi cisternae are optically resolvable, allowing multidimensional imaging studies on cisternal maturation. Amongst the many factors that contribute to maintain the identity and structure of the Golgi is Rab6 (RabC in *A. nidulans*) that, unexpectedly, also appears to play a role in the Spitzenkörper. *rabC*Δ mutants display a marked impairment in apical extension but its most remarkable phenotype is that they have conspicuously fragmented and brefeldin A-insensitive early and late GEs, indicating that RabC contributes to the organisation of the Golgi. As in yeast RacC is involved in the recycling from endosomes to the Golgi of the vacuolar hydrolase receptor $VpsT^{Vps10}$, but not in the traffic of the synaptobrevin homologue SynA between endosomes and the PM, *tlg2*Δ mutants grow normally but are synthetically lethal with *rabC*Δ, indicating that RabC plays Tlg2-independent roles. Our data underscore how wanting our understanding of the complexities of membrane traffic in filamentous fungi is, in spite of its major economic impact in Biotechnology and Medicine.

ChIP-chip/ChIP-seq: protein interactions with DNA (Michell/Freitag) **Nautilus**

Integration of ChIP-Chip/Seq data in a systems biology framework. Marc Facciotti, Elizabeth Wilbanks and David Larsen. UC Davis

The overarching goal of systems biology is the synthesis of biological information into holistic models of cellular physiology. One critical endeavor is to decipher the structure of the regulatory networks that link environmental information to the genome. ChIP-chip and ChIP-Seq are powerful experimental tools for mapping the physical in vivo associations between transcription factors and their cognate promoters, thus for deciphering these regulatory networks. We present some technical considerations regarding the processing of ChIP-Seq data and strategies for the integration of such data in a more general systems biology framework.

Histone H3 de-methylases are involved in regulating primary and secondary metabolism. Agnieszka Gacek[1], Yazmid Reyes-Domínguez[1], Michael Sulyok[2], and Joseph Strauss[1] [1]Fungal Genetics and Genomics Unit, AIT-Austrian Institute of Technology and BOKU University, Vienna, Austria. [2]Christian Doppler Laboratory for Mycotoxin Research, Department IFA-Tulln, BOKU University, Vienna

Opening of chromatin by modification of histone tails is an important process in the synthesis of fungal secondary metabolites (SM). Trimethylation of histone H3 lysine 9 (H3K9me3) and occupancy of heterochromation protein-1(HepA) at this modification site are important marks of transcriptionally silent heterochromatin. In this work we investigate the role de-methylation of H3K9me3 plays in regulating secondary metabolism in *Aspergillus nidulans*. Our Chromatin Immunoprecipitation (ChIP) data provides evidence that both putative Jumonji C-family de-methylases present in the genome are involved in removing the methylation mark from H3K9me3. Deletion of one of the two genes repressed sterigmatocystin (ST) production and the expression of *aflR*, the main regulator of the ST gene cluster. Surprisingly, deletion of both de-methylases restored *aflR* gene expression, but not ST production. Metabolic and transcriptome analysis of the de-methylase mutants suggest that restoration of *aflR* expression is a consequence of de-regulation of primary metabolism, mainly affecting carbon utilization. ST production itself was not restored due to perturbations in primary metabolism presumably affecting precursor provision. Both, de-methylases and LaeA, the conserved global regulator of secondary metabolism, are required to replace the repressing methylation marks on H3 by activating marks.. These results are the first to provide evidence about the role of histone de-methylases in chromatin remodeling, primary metabolism, and secondary metabolism of *A.nidulans*.

Regulatory networks that control morphology and virulence in *Histoplasma capsulatum*. Sinem Beyhan, Matias Gutierrez, Mark Voorhies and Anita Sil, University of California, San Francisco, San Francisco, CA 94143

Histoplasma capsulatum is a dimorphic fungal pathogen that causes respiratory and systemic infections in humans. *H. capsulatum* switches its growth program from an infectious mold (hyphal) form in the soil to a pathogenic yeast form in mammalian hosts. Infection occurs when hyphal fragments are inhaled by the human host. Once inside the host, the pathogen converts to a budding-yeast form, which survives and replicates within host macrophages. Under laboratory conditions, this morphological switch is recapitulated by changing the temperature of the growth environment from room temperature (25˚C) to stimulate hyphal growth to human body temperature (37ºC) to stimulate yeast-form growth. This observation was utilized by our laboratory to identify three genes (*RYP1,2,3*) that are required for yeast-phase growth in response to temperature. *ryp* mutants grow constitutively in the filamentous mold form even at 37ºC. In wild-type cells, *RYP1,2,3* transcripts and proteins accumulate preferentially at 37ºC. In this study, we utilized whole- genome transcriptional profiling and ChIP-chip (chromatin immunoprecipitation-microarray) analysis to identify targets of Ryp1,2,3. Additionally, we performed coimmuniprecipitation to test whether Ryp1,2,3 form a complex. Our findings suggest that (1) Ryp1,2,3 regulates similar and distinct sets of genes; (2) Ryp2 and Ryp3 physically interact; and (3) a transcription factor FacB, which is a target of Ryp1,2,3, regulates the hyphal-to-yeast transition.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Identification of Kinetochore-Like Regions using ChIP-Seq and chromosome segregation analyses

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Chromatin immunoprecipitation – sequencing (ChIP-Seq) has emerged as an efficient tool in yeast to study protein-DNA interactions with high sensitivity and resolution. We are currently performing multiplex ChIP-Seq on a variety of transcription factors and other DNA-binding proteins. Using this technique, we have determined the binding profiles for various kinetochore components, including Cse4, Mif2, Ndc10 and Ndc80. Cse4 binding sites overlap open chromatin sites such as Sono-Seq regions, Pol II binding sites and promoters. Some of these sites (~30), called Kinetochore-Like Regions (KiLRs), are bound by other kinetochore proteins, depending on the genetic background. In plasmid segregation assays, a few KiLRs show increased plasmid retention and a shorter doubling time in selective media than an ARS plasmid, suggesting some centromeric activity, but they are still less efficiently retained than a plasmid containing a bona fide CEN. To study whole-chromosome segregation, we have followed daughter cell budding after galactose inactivation of conditional centromeres. KiLR strains have a higher percentage of budded first daughter cells than WT, suggesting a partial rescue of the inactive centromere. These studies have important implications in the origin and evolution of centromeres.

Regulatory networks during cell-cell communication and germling fusion in *Neurospora crassa*. Abby Leeder, Jingyi Li, Javier Palma Guerrero, and N. Louise Glass. Department of Plant & Microbial Biology, University of California, Berkeley, 94720

Cell fusion between genetically identical cells occurs in most multicellular organisms, from simple ascomycete fungi to mammals. In fungi, fusions between identical conidia have long been observed and are thought to provide many benefits to the hyphal network, such as allowing for efficient resource sharing. Prior to fusion, pairs or groups of cells must first recognize each other and then undergo communication and directed growth until cell wall contact is made. *Neurospora crassa* germlings alternate between two different physiological states during chemoattraction, in order to both send and receive communication signals. This system allows bidirectional interactions between cells and also avoids self-stimulation, which is one of the largest obstacles associated with communication between developmentally equivalent, genetically identical cells. We have previously determined that the kinase MAK-2 is directly involved in germling fusion, and that it shows dynamic localization during the communication process. Current work is focusing on the regulatory networks that involve MAK-2 and/or it's downstream effector PP-1, a homolog of the transcription factor STE12. Using various analyses, we have determined novel proteins important for cell communication and fusion. These networks and proteins will be discussed here.

Characterization of Circadian Clock Output Pathways Regulated by Adv-1 in Neurospora crassa Using Chip-seq. Rigzin Dekhang[1], Kristina M. Smith[2], Erin L. Bredeweg[2], Jillian M. Emerson[3], Matthew S. Sachs[1], Jay C. Dunlap[3], Michael Freitag[2], and Deborah Bell-Pedersen[1]. [1]Department of Biology, Texas A&M University, College Station, TX; [2]Program for Molecular and Cellular Biology, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331; [3]Department of Biology, Dartmouth Medical School, Hanover, NH.

Circadian clocks are biological timekeeping mechanisms used by phylogenetically diverse organisms to control the rhythmic expression of genes involved in physiology, metabolism and behavior. In Neurospora crassa the blue light photoreceptor and PAS-domain GATA transcription factor (TF) WC-1 interacts with another PAS-domain GATA TF WC-2 to form the White Collar Complex (WCC). In the clock, the WCC functions as the positive element in the FRQ/WCC oscillator and it signals time-of-day information through the output pathways to control the expression of a large number of clock-controlled genes (ccgs). ChIP-seq of WC-2 identified hundreds of targets of the WCC, including the promoters of 24 TFs. These TFs are thought to regulate the expression of second tier targets of the clock. One of these TFs, ADV-1, is expressed with a circadian rhythm. Inactivation of the adv-1 gene abolishes the circadian rhythm in conidiation, but does not alter the level or activity of the FRQ/WCC oscillator components. Taken together, these data suggest that the ADV-1 functions within an output pathway from the clock. Results from ChIP-seq and RNA-seq to identify the direct and indirect targets of ADV-1 will be discussed.

ChIP sequencing reveal dual role for the transcription regulator Tri6 in the phytopathogen *Fusarium graminearum*. Charles G. Nasmith [1,2] Li Wang[1,2], Sean Walkowiak[1,2], Yunchen Gong[3], Winnie Leung[1], David S. Guttman[3], Gopal Subramaniam[1] [1]Agriculture and Agri-Food Canada, 960 carling Avenue, Ottawa, Ontario, Canada, K1A 0C6 [2]Contributed equally [3]CAGEF, University of Toronto, 25 Willcocks St. Toronto, Ontario, Canada, M5S 3B3 Email: subramaniamra@agr.gc.ca

The synthesis and accumulation of the trichothecene 15-acetyldioxynivalenol (15-ADON) is associated with Fusarium head blight (FHB) disease of cereal crops. Activation of the trichothecene gene cluster in the phytopathogen *Fusarium graminearum* requires the transcriptional regulator Tri6. Genome wide chromatin immunoprecipitation (ChIP) sequencing revealed targets of Tri6 both within and outside the trichothecene gene cluster. Bioinformatics analysis of the promoters of the targets established a consensus binding site for Tri6. The electro mobility shift analysis (EMSA) in addition to confirming the consensus binding site, but also identified another binding site specifically enriched in the promoters of the genes involved in secondary metabolism.

Using Chip-Seq to Disect Microbial Regulatory Networks. James Galagan, Boston University and the Broad Institute, Massachusetts

Chromatin immunoprecipitation followed by sequencing (ChIP-seq) has emerged as a powerful tool for genome-wide mapping of the binding sites of DNA binding proteins. Although many published ChIP-seq projects have focused on mammalian and other larger genomes, ChIP-seq is particularly powerful when applied to relatively smaller microbial genomes. Owing to the large number of reads currently produced by next generation sequencing instruments, ChIP-seq on microbial genomes can produce binding site predictions with extraordinarily high sensitivity and spatial resolution. This in turn enables us to probe the biophysics of binding, the architecture of individual promoters, and the structure of regulatory binding networks.
In my talk I will describe our experience in using ChIP-seq to comprehensively map the transcription factor binding network of microbial genomes. I will highlight our experience in *Mycobacterium tuberculosis*, and discuss ongoing applications to *Neurospora*. In particular, I will discuss (1) the lessons learned with respect to ChIP methodology for microbial genomes, (2) the tools we have developed for analyzing and visualizing high-throughput ChIP-seq data, (3) the integration with transcription profiling data, and (4) the observations we are making with respect to transcription factor binding and regulatory networks.

Education and Public Outreach (Pukkila/Zolan) **Scripps**

The organizers have indicated that abstracts were not requested for this session.

Concurrent Sessions III

Cell Cycle, Development, and Morphology (Talbot/Riquelme) **Fred Farr Forum**

RNA is asymmetrically localized in *Aspergillus fumigatus*. Ken Oda, Susan Cowden, Mara Couto, John Kerry and Michelle Momany. Department of Plant Biology, University of Georgia, Athens, GA 30602 USA.

The generation of asymmetry, or polarity, is important for organisms ranging from unicellular yeasts to multicellular plants and animals. But, perhaps the most extreme examples of polar growth are found within the filamentous fungi. When dormant conidia of *Aspergillus fumigatus* are inoculated to medium containing carbon, they synchronously break dormancy, expand isotropically, and establish an axis of polarity where the primary germ tube will emerge. We took advantage of this synchronous early development to analyze temporal and spatial gene expression patterns during the isotropic to polar switch. Microarray analysis of cells undergoing the transition from isotropic to polar growth showed very little change in the expression of most genes. In contrast, laser microdissection and deep sequencing showed large differences in the localization of individual mRNAs in different regions of young hyphae. *In situ* hybridization with selected highly expressed genes as probes confirmed asymmetric RNA localization along the hypha. momany@plantbio.uga.edu

Macroautophagy-mediated Degradation of Whole Nuclei in the Filamentous Fungus *Aspergillus oryzae*. Jun-ya Shoji, Takashi Kikuma, Manabu Arioka, and Katsuhiko Kitamoto Department of Biotechnology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan email; junya@fungalcell.org

Filamentous fungi consist of continuum of multinucleate cells called hyphae, and proliferate by means of hyphal tip growth. Accordingly, research interest has been focusing on hyphal tip cells, but little is known about basal cells in colony interior that do not directly contribute to proliferation. Here, we show that autophagy mediates degradation of basal cell components in the filamentous fungus *Aspergillus oryzae*. In basal cells, enhanced green fluorescent protein (EGFP)-labeled peroxisomes, mitochondria, and even nuclei were taken up into vacuoles in an autophagy-dependent manner. During this process, crescents of autophagosome precursors matured into ring-like autophagosomes to encircle apparently whole nuclei. The ring-like autophagosomes then disappeared, followed by dispersal of the nuclear material throughout the vacuoles, suggesting the autophagy-mediated degradation of whole nuclei. We also demonstrated that colony growth in a nutrient-depleted medium was significantly inhibited in the absence of functional autophagy. This is a first report describing autophagy-mediated degradation of whole nuclei, as well as suggesting a novel strategy of filamentous fungi to degrade components of existing hyphae for use as nutrients to support mycelial growth in order to counteract starvation.

Comparative analysis of hyphal Ca^{2+} dynamics in three *Fusarium* species and the role of calcium channel genes in the generation of hyphal tip Ca^{2+} pulses. Hye-Seon Kim[1,2], Kirk Czymmek[2] and Seogchan Kang[1]. [1]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802,USA; [2]Department of Biological Sciences, University of Delaware, Newark, DE 19716,USA. E-mail: hxk234@psu.edu

Calcium is a universal messenger that directs an array of diverse cellular and developmental processes in response to external stimuli. Pulsatile cytoplasmic calcium ($[Ca^{2+}]_C$) signatures, generated by combined action of several types of channels in the plasma and organellar membranes, are believed to translate external stimuli to specific cellular responses through the well-conserved calcium signaling pathway. However, visualization of subcellular $[Ca^{2+}]_C$ dynamics in yeasts and filamentous fungi has been difficult due to technical challenges associated with probes used for imaging $[Ca^{2+}]_C$. Previously, we transformed two fungi, *Fusarium oxysporum* and *Magnaporthe oryzae*, with Cameleon (YC3.60) and imaged dynamic $[Ca^{2+}]_C$ in relation to specific stimuli and key growth- or development-related events such as branching, septum formation, and cell-cell contact. We successfully expressed Cameleon in the cytoplasm of three other *Fusarium*species, including *F. graminearum*, *F. verticilliodes*, and *F. solani*. A comparison of temporal and spatial dynamics of $[Ca^{2+}]_C$ among *Fusarium* species revealed that all species showed tip high $[Ca^{2+}]_C$ but time-lapse ratiometric analysis revealed apparent species-specific pulsatile patterns. Furthermore, in order to better understand which calcium channels play a role in generating pulsatile $[Ca^{2+}]_C$ signatures, three channel genes were specifically disrupted in *F. graminearum*. Taken together, this study provided important clues on fundamental aspects of subcellular $[Ca^{2+}]_C$ signaling in filamentous fungi.

DOI: 10.4148/1941-4765.1066

Investigating the role of the septin gene family in *Magnaporthe oryzae* during rice infection. Yasin F. Dagdas, Min He, Michael J. Kershaw and Nicholas J. Talbot**.** School of Biosciences, University of Exeter, Geoffrey Pope Building, Exeter EX4 4QD, United Kingdom. email: N.J.Talbot@exeter.ac.uk

Magnaporthe oryzae is the causal agent of rice blast, one of the most serious economic problems affecting rice production. During plant infection, *M. oryzae* develops a differentiated infection structure called an appressorium. This unicellular, dome-shaped structure generates cellular turgor, that is translated into mechanical force to cause rupture of the rice cuticle and entry into plant tissue. We have shown that development of a functional appressorium requires completion of mitosis and initiation of autophagic recycling of the contents of the fungal spore to the appressorium. Elaboration of the appressorium also involves an unusual septation event in which the site of nuclear division is spatially uncoupled from the position of cytokinesis. To understand the morphological transitions associated with plant infection, we have been investigating the septin gene family in *M. oryzae*. Septin mutants are defective in pathogenesis and show a variety of morphological aberrations, including defects in appressorium function and the development of invasive hyphae. We have also observed that a septin ring is associated with the appressorium pore and the re-establishment of polarity at the base of a functional appressorium.

RNA-binding protein mediates *Magnaporthe oryzae* Cellular Differentiation and Plant Infection through Regulation of TOR signaling cascade. Marina Franceschetti [1], Emilio Bueno[1], Richard A. Wilson[2], Grant Calder[1] and Ane Sesma[1]. [1]John Innes Centre, Colney lane, Norwich, United Kingdom (ane.sesma@bbsrc.ac.uk) [2] University of Nebraska, Lincoln, USA

The RBP35 protein contains one RNA Recognition Motif (RRM) and six Arg-Gly-Gly tripeptides and is required for *Magnaporthe oryzae* plant invasion. The RRM motif is by far the most common and best characterized of the RNA- binding modules and can function in all post-transcriptional gene-expression processes. RBP35 homologues are found only in filamentous fungi. Western blots identified two RBP35 protein isoforms, the expected full length protein (RBP35a, 44 kDa), and a smaller protein (RBP35b, 31 kDa) that derives from the proteolytic cleavage of RBP35a. Both isoforms bind poly$(G)_{30}$ RNA homopolymers exclusively and show a steady-state nuclear localization. FRAP experiments suggest the ability of RBP35 to form different protein complexes in the nucleus of conidia and appressoria. Truncated and mutated protein variants of RBP35 accumulate in cytoplasmic granules, possibly processing bodies and/or stress granules, indicating an involvement of RBP35 in translational repression of targeted mRNAs. Comparative transcriptome analysis reveal that several signaling pathways are altered in the *rbp35* mutant including the target of rapamycin (TOR), a conserved Ser/Thr kinase that regulates cell growth and metabolism in response to environmental cues. We have optimized tandem affinity purification experiments in order to identify RBP35-interacting proteins and direct mRNA targets. Results of the pull-down experiments using RBP35-HA-FLAG protein fusions will be presented.

The velvet-like complex from chrysogenum: A regulatory network of five subunits controls secondary metabolism and morphogenesis. Kück U[1], Hoff B[1], Kamerewerd J[1], Kopke K[1], Wolfers S[1], Katschorowski A[1], Milbredt S[1], Koutsantas K[1], Kluge J[1], Zadra I[2], Kürnsteiner H[2] [1]Christian Doppler Laboratory for Fungal Biotechnology, Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Universitätsstraße 150, 44780 Bochum; [2]Sandoz GmbH, 6250 Kundl, Austria

The global regulator velvet together with laeA is a core component of the velvet complex from Aspergillus nidulans. We have characterized a velvet-like complex from the penicillin producer Penicillium chrysogenum, which contains at least five different subunits. Included is PcvelA P. chrysogenum, which is an inhibitor of light-dependent conidiation and affects the biosynthesis of the beta-lactam antibiotic penicillin*. We will present an extensive analysis of subunits PcvelB, PcvelC and PcvosA of the velvet-like complex, including data from array hybridization, high performance liquid chromatography, quantification of penicillin titers, microscopic investigations and mass spectrometry. We provide evidence that all subunits of this complex have conserved as well as novel roles in secondary metabolism and morphogenesis in P. chrysogenum. These results confirm and extend the current picture of regular networks controlling both, fungal secondary metabolism and morphogenesis. *Hoff et al. EUKARYOTIC CELL 9: 1236–1250 (2010)

Regulatory networks coordinating nuclear division and pathogenic development in *Ustilago maydis*. Kai Heimel, Mario Scherer, Miroslav Vranes, David Schuler, and Jörg Kämper. Department of Genetics, Karlsruhe Institute of Technology, 76187 Karlsruhe, Germany

In the smut fungus *Ustilago maydis*, pathogenic and sexual development are orchestrated by the *a* and *b* mating-type loci. Activation of either mating-type locus triggers a cell cycle arrest as a prerequisite for the formation of the infectious dikaryon, which is released only after penetration of the host plant. *b* encodes the heterodimeric transcription factor bE/bW which coordinates a regulatory network consisting of different transcription factors: Rbf1, as a master regulator, is required for the expression of most *b*-regulated genes, including the transcription factors Hdp2 and Biz1. Rbf1 expression is sufficient to initiate pathogenic development, but further development requires (1) additional factors as the *b*-dependently expressed Clp1 protein for cell cycle progression and (2) additional *b*-regulated genes to establish the biotrophic interface. Clp1 interacts physically with bW and blocks *b*-dependent functions, such as the *b*-dependent G2 cell cycle arrest, dimorphic switching and pathogenic development. The interaction of Clp1 with Rbf1 leads to repression of the *a*-dependent pheromone pathway, resulting in a release of the *a*-induced cell cycle arrest. Thus, the concerted interaction of Clp1 with Rbf1 and bW coordinates *a*- and *b*-dependent cell cycle control to ensure cell cycle release and progression at the onset of biotrophic development. Another level of complexity is added by the action of Biz1. Similar to bE/bW or Rbf1, induction of Biz1 leads to a cell cycle arrest; in addition, Biz1 is required for the regulation of various genes during the early infection phase. The advantage of such complex regulatory pathways is the dynamic integration of different signals to control developmental decisions, as, for example, the adaptation to specific tissues.

Identification of a microtubule associating protein that interacts with nuclear pore complex proteins during mitosis. Nandini Shukla, Aysha H. Osmani, Stephen A. Osmani. The Ohio State University, Columbus, OH, USA. (shukla.25@buckeyemail.osu.edu)

Aspergillus nidulans exhibits partially open mitosis wherein the nuclear pore complexes (NPCs) undergo partial disassembly similar to the initial stages of open mitosis of higher eukaryotes. It is currently not known how the partial disassembly and re-assembly of NPCs is carried out or regulated. One potential mechanism is the existence of NPC disassembly factors that would bind preferentially to NPC proteins (Nups) during mitosis to promote their disassembly from the core structure. The current work describes efforts to identify such proteins using affinity purification - MS analyses of Nups from interphase and mitotic cells. An interesting novel protein, ANID_03906, identified by this approach preferentially co-purifies with mitotic Gle1, Nup133, and Mad1. Endogenous GFP tagging revealed that ANID_03906, locates to cytoplasmic microtubules during interphase. It appears to both coat microtubules and form mobile foci that move along microtubules. Drug treatment to depolymerize microtubules dramatically modifies the location of ANID_03906 which locates to immobile aggregates without microtubule function. These data suggest this previously unstudied protein might play a role during interphase involving microtubules and at mitosis involving specific Nups. Future work aims at understanding the interactions of ANID_03906 with Nups and components of the cytoskeleton during cell cycle progression. (Supported by NIH grant GM042564)

Host Selective Toxins (Turgeon/Friesen) **Nautilus**

Comparative genomics of Host Selective Toxin-producing Cochliobolus pathogens of cereals. B. Gillian Turgeon, Bradford Condon, and Dongliang Wu Dept. of Plant Pathology & Plant-Microbe Biology, Cornell University, bgt1@cornell.edu

An overview of how we are using the fungal genomics resources, in partnership with the Joint Genome Institute's Fungal Genomics Program (http://genome.jgi-psf.org/programs/fungi/index.jsf), to understand evolution and function of genes responsible for production of Host Selective Toxins (HST), will be presented. Many Dothideomycete genome sequences are available and more are underway under the auspices of the JGI program. HST- producing members of the genus Cochliobolus include the maize pathogens *C. heterostrophus* (Southern Corn Leaf Blight), which produces the HST, T-toxin, and *C. carbonum* (Leaf Spot), which produces HC-toxin, and the oat pathogen, *C. victoriae* (Victoria Blight), which produces victorin. Case studies illustrating how the availability of these genome sequences facilitates a better understanding of each pathosystem and Dothideomycete interactions with plants, in general, will be discussed.

A 'Born Again' fungal virulence effector. Thomas J. Wolpert, Department of Botany and Plant Pathology and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97330.

Cochliobolus victoriae is the causal agent of a disease called Victoria Blight. The fungus is pathogenic because of its production of the host-specific toxins collectively referred to as victorin. Only isolates that produce victorin are pathogenic and only on hosts that are sensitive to the toxin. Toxin sensitivity and consequently, disease susceptibility in the host is conditioned by a single dominant gene. Thus, Victoria Blight conforms to the gene-for-gene paradigm except that the phenotypes associated with the host and pathogen are inverted from those classically observed. This genetic pattern suggests that the pathogen could be exploiting the defense response to achieve virulence. Results to be discussed support this contention and imply that vicotrin, or a victorin-like molecule, originally evolved as a virulence effector which was subsequently "defeated" through host recognition to become an avirulence effector, Ironically, precisely because of its recognition and ability to incite defense, this molecule was adapted/mimicked by *Cochliobolus victoriae* to once again function as a virulence effector.

Pathogenicity chromosomes in host-specific toxin-producing *Alternaria* species. Motoichiro Kodama[1,2], Yasunori Akagi[1], Kazumi Takao[1], Yoshiaki Harimoto[3] and Takashi Tsuge[3]. [1]Laboratory of Plant Pathology, and [2]Fungus/Mushroom Resource and Research Center, Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan, [3]Nagoya University, Japan, mk@muses.tottori-u.ac.jp

Alternaria alternata plant pathogens consist of seven variants (pathotypes), all of which produce host-specific (selective) toxins (HSTs); all cause necrotic diseases on different plants. We have shown that all strains of *A. alternata* pathotypes harbour small and extra chromosomes, whereas nonpathogenic isolates do not have these small chromosomes. Based on biological and pathological observations, those small chromosomes were termed conditionally dispensable chromosomes (CDCs) and pathogenicity chromosomes. HST biosynthetic genes have been isolated from five pathotypes (apple, Japanese pear, strawberry, tangerine, and tomato) of *A. alternata* and found to be clustered on the CDCs. Sequencing of the entire CDCs of the apple, strawberry and tomato pathotypes which produce AM-, AF- and AAL-toxins, respectively, revealed that the CDC of each consists of CDC-specific and repetitive sequences related to the HST production and pathogenicity. The CDC in the tomato pathotype strains from different geographical origins was identical although the genetic backgrounds of the strains differed. The results imply that CDCs have a different evolutionary history from the essential or core chromosomes in the same genome. A hybrid strain between two different pathotypes was shown to harbour the CDCs from both parental strains and had an expanded pathogenicity range, indicating that CDCs could be transmitted from one strain to another and stably maintained in the new genome. We propose a hypothesis whereby the ability to produce HSTs and to infect a plant is distributed among *A. alternata* strains by horizontal transfer of an entire pathogenicity chromosome (CDC). This could provide a possible mechanism by which new pathogens arise in nature.

Histone deacetylase inhibitor HC-toxin from *Alternaria jesenskae*.Wanessa D. Wight[1,2] and Jonathan D. Walton[1,2]. [1] Department of Energy - Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, U.S.A.[2] Cell and Molecular Biology Program, Michigan State University, East Lansing, MI 48824, U.S.A. wightwan@msu.edu

HC-toxin is a cyclic tetrapeptide with known histone deacetylase (HDAC) inhibition activity. It is an essential virulence factor for the maize pathogen *Cochliobolus carbonum*. Biosynthesis of HC-toxin is controlled by a complex genetic locus, *TOX2*, that spans >500 kb. *TOX2* contains at least seven genes including the four-domain nonribosomal peptide synthetase, HTS1. All of the TOX genes are present in two to three copies at the *TOX2* locus. *Alternaria jesenskae* (1), also produces HC-toxin (R. Labuda, personal communication). A genome survey sequence of *A. jesenskae* was generated by 454 pyrosequencing. Unambiguous orthologs of all seven known genes involved in HC-toxin biosynthesis from *C. carbonum* were identified in *A. jesenskae*. The average percent identities of the TOX genes from the two fungi range from 80 to 85%. As in *C. carbonum*, many of the HC-toxin genes are present in two copies in *A. jesenskae*. Variation in the genomic organization of the TOX genes in addition to the high degree of similarity among housekeeping genes suggest that the HC-toxin clusters in the two fungi might be the result of evolution from a common ancestor and not the result of horizontal gene transfer event. 1.Labuda R., Eliás P. Jr., Sert H., Sterflinger K. 2008. *Alternaria jesenskae* sp. nov., a new species from Slovakia on *Fumana procumbens* (Cistaceae). Microbiol Res.163(2):208-14

Same Fungus, Two Different Host-Selective toxins: Perceptions and Outcomes.
L.M. Ciuffetti, V.M. Manning, I. Pandelova, and M. Figueroa, Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA

Host-selective toxins (HSTs) are pathogenicity/virulence factors produced by a group of necrotrophic fungal pathogens. Data support that these disease interactions often follow an inverse gene-for-gene interaction where a single locus in the host is responsible for toxin sensitivity. The long-term goal of the research conducted in our lab is to fully describe the molecular interactions of the HSTs produced by the fungus, *Pyrenophora tritici-repentis*, with its host plant, wheat. These studies include the identification and characterization of genes involved in pathogenicity and host specificity, the mechanisms by which this fungus acquires these virulence factors, the structural requirements responsible for activity, and the determination of the molecular site- and mode-of-action of these HSTs. Our studies have focused on Ptr ToxA and Ptr ToxB, two proteinaceous HSTs of *P. tritici-repentis*. Although these two HSTs appear to confer pathogenicity through distinctly different mechanisms, they also share commonalities. The ability of a pathogen to produce different pathogenicity factors that promote cell death by a variety of mechanisms provides a unique opportunity to investigate and unravel the elements of disease susceptibility. The perceptions and outcomes of these two HSTs with the host will be the focus of this presentation.

A novel, cysteine-rich fungal effector triggers light-dependent susceptibility in the wheat-*Stagonospora nodorum* interaction.
Zhaohui Liu[1], Zengcui Zhang[1], Justin D. Faris[2], Richard P. Oliver[3], Peter S. Solomon[4], Timothy L. Friesen[1,2] [1]Department of Plant Pathology, North Dakota State University, Fargo, ND; [2] Northern Crop Science Lab, USDA-ARS, Fargo, ND; [3]Department of Environment & Agriculture, Curtin University, Perth, Australia; [4]School of Biology, The Australian National University, Canberra, Australia.

SnTox1 was the first necrotrophic effector identified in *S. nodorum*, and was shown to induce necrosis on wheat lines carrying *Snn1*. To isolate the SnTox1-encoding gene, we used bioinformatics tools followed by heterologous expression in *Pichia pastoris*. *SnTox1* encodes a 117 aa protein with the first 17 predicted as a signal peptide, and strikingly, the mature protein contains 16 cysteines. The transformation of *SnTox1* into an avirulent isolate was sufficient to make the strain pathogenic. Additionally, the deletion of *SnTox1* in virulent isolates renders the *SnTox1* mutant nonpathogenic on the *Snn1* differential line. The SnTox1-Snn1 interaction involves an oxidative burst, DNA laddering, and defense gene expression, all hallmarks of programmed cell death. In the absence of light, SnTox1-induced necrosis is blocked along with the disease development induced by the SnTox1-Snn1 interaction. By comparing the infection processes of a GFP-tagged avirulent isolate and the same isolate transformed with *SnTox1*, we conclude that SnTox1 plays a critical role in the initial penetration and subsequent proliferation in the host. This research provides important insights into the molecular basis of the wheat-*S. nodorum* interaction, a model for necrotrophic pathosystems.

A proteomics approach to dissect SnToxA effector mode-of-action in wheat Delphine Vincent[1], Ulrike Mathesius[1], Richard Lipscombe[2], Richard P. Oliver[3], Peter S. Solomon[1] [1]Research School of Biology, College of Medicine, Biology and Environment, The Australian National University, ACT Australia; [2]Proteomics International, Perth, WA, Australia; [3]Curtin University of Technology, Perth, WA, Australia delphine.vincent@anu.edu.au

Stagonospora nodorum is a necrotrophic fungal pathogen of wheat causing devastating foliar damage resulting in significant yield losses globally. *S. nodorum* operates in inverse gene-for-gene manner through the interaction of a secreted effector and a dominant host susceptibility protein resulting in disease development. The effector protein SnToxA and its corresponding host gene Tsn1 follow such a system. This study aims at deciphering the molecular responses triggered by SnToxA in the wheat (*Triticum aestivum*) susceptible cultivar BG261 over a 0-48hrs time course using a gel-based proteomics strategy. Wheat leaves were infiltrated with SnToxA and sampled at 0, 0.5, 4, 12, 24, and 48 hrs post-infiltration (hpi). Both acidic and basic proteins were studied and they generally display an up- regulation at 12 hpi followed by a down-regulation at 48 hpi. Differentially-expressed proteins are predominantly involved in energy and protein metabolisms, with many of the proteins identified localised in the chloroplast. The identities of these proteins and their possible roles will be discussed.

Quantitative variation in activity of ToxA haplotypes from *Stagonospora nodorum* and *Pyrenophora tritici-repentis* refines the distinction between biotrophic and necrotrophic interactions. Kar-Chun Tan[1], Margo Ferguson-Hunt[2], Kasia Rybak[2], Ormonde D. C. Waters[1], Will A. Stanley[3], Charles S. Bond[4], Eva H. Stukenbrock[5], Timothy L. Friesen[6], Justin D. Faris[6], Bruce A. McDonald[7] and Richard P. Oliver[1*] . [1]Environment and Agriculture, Curtin University, Bentley WA 6102, Australia. [2]Health Science, Murdoch University, Murdoch, WA6150, Australia. [3]ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, Crawley 6009 WA, Australia. [4]Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley 6009 WA, Australia. [5]Max Planck Institute Marburg, Karl von Frisch Str. 10, D-35043 Marburg, Germany. [6]USDA-ARS Cereal Crops Research Unit, Red River Valley Agricultural Research Center, Fargo, ND 58105. [7]Plant Pathology Group, Institute of Integrative Biology, ETH Zurich, Universitätsstr 2, CH-8092 Zurich, Switzerland

The parallels between host-specific toxins produced by necrotrophic pathogens (we now prefer to call them necrotrophic effectors (NEs)) and avirulence gene products produced by biotrophs (we suggest the name biotrophic effectors or BEs) have accumulated over the last decade. The effectors produced by both classes of pathogen operate in a species- and cultivar-specific manner and produce reactions in the host that are operationally very similar. In at least three cases, we now know that the host partner (the direct or indirect receptor) for NEs is - like in BEs - an NBS-LRR gene. Nonetheless there is a clear functional difference; recognition of a BE leads to resistance; recognition of the NE leads to virulence. Overall, resistance in biotrophic interactions tends to be qualitative because recognition of a single effector is sufficient to induce resistance. In contrast, resistance in necrotrophic interactions tends to be quantitative; this has assumed to be because multiple effectors interact with multiple receptors and each positive interaction acts quasi-additively to produce the virulence phenotype. High dN/dS ratios have been observed in many BE and R-genes in biotrophic interactions and this has been cited as evidence of diversifying selection associated with qualitative interactions leading to a co-evolutionary arms race. This paper discusses and refines the parallels between NEs and BEs in the light of new data about ToxA.

ToxA is a proteinaceous NE produced by two wheat pathogens, *Stagonospora nodorum* and *Pyrenophora tritici-repentis.* Both pathogens produce several other NEs and resistance is quantitatively inherited. ToxA makes a significant contribution to virulence in interactions of both species with wheat cultivars that carry the NBS-LRR gene *Tsn1*. Seven mature versions of the ToxA protein are encoded by different *ToxA* genes in *S. nodorum* populations while a single version exists in most isolates of *P. tritici-repentis.* The genes exhibit an elevated dN/dS ratio. known sensitive alleles of the gene in bread wheats encode an identical protein.

We expressed and purified 8 versions of ToxA. Circular dichroism spectra indicated that all versions were structurally intact and have indistinguishable secondary structural features. We expect that each variant has a similar tertiary structure. All versions induced necrosis when introduced into any *Tsn1* wheat line. However, we observed quantitative variation in effector activity for the different ToxA variants. The least active version was the one present in isolates of *P. tritici-repentis*. Different wheat lines carrying identical *Tsn1* alleles varied in sensitivity to ToxA. The presence of diversifying selection is often indicative of direct protein-protein effector/receptor interactions leading to a co-evolutionary arms race. If diversifying selection led to the observed *ToxA* diversity, then given that all *Tsn1* alleles are identical, we predict that the gene or genes encoding wheat proteins that interact with ToxA and with the *Tsn1* gene product will also show diversifying selection. An alternative explanation for the observed diversity is directional selection that has favoured *ToxA* alleles encoding higher virulence in regions where *Tsn1* wheat is common. In either scenario, our results indicate that subtle differences in effector alleles may underlie quantitative differences in virulence in gene-for-gene systems.

Fungicides and Antifungals (Cowen/Fillinger) **Chapel**

Control of the Chromosome Acetylation Cycle as a novel anti-fungal therapeutic strategy. Hugo Wurtele[1],*, Sarah Tsao[1], Guylaine Lépine[1], Alaka Mullick[2], Jessy Tremblay[2], Paul Drogaris[1], Eun-Hye Lee[1], Pierre Thibault[1], Alain Verreault[1] and Martine Raymond[1]. [1]Institute for Research in Immunology and Cancer, Université de Montréal, Montreal, Canada. [2]Biotechnology Research Institute, National Research Council of Canada, Montreal, Canada. hugo.wurtele@umontreal.ca

Candida albicans is a fungal pathogen that causes life-threatening infections in immunocompromised individuals, such as AIDS patients and those undergoing cancer chemotherapy. Although pharmacological agents are available to treat C. albicans infections, the emergence of drug-resistant strains and hospital-acquired infections by healthy individuals is a growing concern for healthcare organisations. In yeast, histone H3 lysine acetylation (H3K56ac) is an abundant modification regulated by enzymes that have fungal-specific properties, making them appealing targets for antifungal therapy. We show that H3K56ac in C. albicans is regulated by the Rtt109 acetyltransferase and the Hst3 deacetylase. The absence of H3K56ac sensitises C. albicans to currently employed antifungal agents. Inhibition of Hst3 by conditional gene repression or nicotinamide treatment results in a dramatic loss of cell viability associated with anomalous filamentation and aberrant DNA staining. Using genetic and pharmacological approaches, we demonstrate that inhibition of H3K56 deacetylation reduces virulence in a mouse model of C. albicans infection. Our results suggest that modulation of H3K56ac may prove a valuable target to treat C. albicans and possibly other fungal infections.

Genome-wide screens using a natural product saponin identify three PDR pathway target genes, *PDR19, PDR20 and PDR21,* which influence lipid homeostasis and membrane permeability in *Saccharomyces cerevisiae*. Gary Franke, Daniel Chirinos, Virginia Aberdeen and Scott Erdman. Dept. of Biology, Syracuse University seerdman@syr.edu

To investigate the mechanisms of action of an antimicrobial natural product saponin and to gain insights into lipid and membrane homeostasis in fungi, we carried out two genome-wide screens in yeast to identify genes involved in these cellular processes. A collection of 4,851 viable gene deletion strains was screened for growth rate on medium containing a triterpene glycoside (TTG) saponin. Deletant strains sensitive or resistant to TTGs were identified and collectively were found to be enriched for genes involved in several cellular processes, including lipid metabolism, cell wall assembly and toxin resistance. This screen identified many known, previously known and novel non-essential yeast genes whose absence affects growth under lipid and membrane disturbing conditions. A high copy plasmid suppression screen of one significantly TTG-sensitive mutant was also performed to learn more about TTG effects and potential mechanisms of resistance. This approach identified 11 different high-copy suppressors operating mainly through three pathways: vesicle trafficking, stress responses and the pleiotropic drug resistance (PDR) response. Analyses of the antifungal drug and chemical sensitivities of deletion strains for a subset of these high copy suppressors demonstrate them to be members of a novel group of PDR target genes, *PDR19, PDR20 and PDR21,* with specific roles in lipid and membrane homeostasis functions. Physiological studies of cells lacking these genes demonstrate their roles in influencing plasma membrane permeability in both normal and drug treated cells. Supported by NSF grants: SGER #0222591 and NSF #0315946

Characterization of Fluconazole-related Chromosomal duplication in *Cryptococcus neoformans*. Popchai Ngamskulrungroj, Yun Chang and Kyung J. Kwon- Chung Molecular Microbiology Section, Laboratory of Clinical Infectious Diseases, NIAID, NIH, Bethesda, Maryland, USA Email: ngamskulrungrp@niaid.nih.gov

Cryptococcus neoformans, a basidiomycetous yeast, causes opportunistic infection mainly in HIV patients worldwide. Fluconazole (FLC), an antifungal triazole drug, has been the drug of choice for the treatment of cryptococcosis and fluconazole therapy failure cases have been increasingly reported. Recently, an intrinsic mechanism of adaptive resistance to triazoles termed heteroresistance (HR) was characterized in *C. neoformans*. Heteroresistance was defined as the emergence of a resistant minor subpopulation that could tolerate concentrations of FLC higher than the strain's MIC. The lowest concentration of fluconazole at which such a population emerged was defined as its LHF (level of heteroresistant to fluconazole). These resistant subpopulations were found to contain disomic chromosomes (Chr). Only Chr1 was found to be duplicated in the populations that grew at their LHF. However, additional duplications involving Chr4, Chr10 and Chr14 were observed as the drug concentration was increased. The roles of *ERG11*, the major target of FLC, and *AFR1*, an ABC transporter with FLC specificity, were found to be important for Chr1 duplication. However, the factors affecting duplication of the other chromosomes have not yet been identified. Since FLC is known to cause perturbation of the cell membrane and is effluxed by various ABC transporters, nine genes on Chr4 that are putatively associated with such functions were disrupted by biolistic transformation. Regardless of their impacts on FLC susceptibility, disruptions of the homologs *SEY1*, a GTPase, *GLO3* and *GCS1*, the ADP-ribosylation factor GTPase activating protein, reduced the frequency of Chr4 duplication. In addition, deletion of a *YOP1* homolog, which is known to interact with *SEY1* and located on Chr7, also reduced the Chr4 duplication frequency. This suggests that the function of these genes is important for duplication of Chr4 under FLC stress in *C. neoformans*.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Natural and acquired fenhexamid resistance in *Botrytis* spp.: What's the difference? Alexis Billard[1], Sabine Fillinger[1], Pierre Leroux[1], Jocelyne Bach[1], Pauline Solignac[1], Catherine Lanen[1], Hélène Lachaise[2], Roland Beffa[2], and Danièle Debieu[1] [1]BIOGER CPP, INRA Versailles-Grignon, France; [2]Bayer Cropsciences, La Dargoire Research Station, Lyon, France - debieu@versailles.inra.fr

Antifungal compounds such as ergosterol biosynthesis inhibitors are widely used to control crop diseases. Among them, one of the most recent, the hydroxyanilide fenhexamid, is efficient principally against *Botrytis cinerea*, the major causal agent of grey mould. Fenhexamid is a new type of ergosterol biosynthesis inhibitor affecting the sterol C4 demethylation processes due to its specific interaction with one of the four proteins of the enzymatic complex, the 3-keto reductase. Our regular monitoring conducted on French vineyards allowed the identification of the first isolates of *B. cinerea* with acquired resistance. Two types of resistant isolates named HydR3$^-$ and HydR3$^+$ were distinguished by their resistance level. This acquired resistance is due to point mutations in the erg27 gene leading to target modifications. These modifications induce a reduced in affinity of fenhexamid towards its target, the 3-keto reductase. Because of their high resistant level, the HydR3$^+$ strains have to be considered relative to the risk of resistance phenomenom occurrence in vineyards. Fitness studies conducted *in vitro* on isogenic mutants showed altered "overwintering" capacities of HydR3$^+$ mutants suggesting that they probably do not impact fenhexamid's field efficacy. While *B. cinerea's* acquired resistance could be explained only by target modifications, as in most cases of fungicide resistance, the situation is different for the related species *Botrytis pseudocinerea* naturally resistant to fenhexamid. We show that *erg27* polymorphism only slightly contributes to resistance whereas fenhexamid detoxification by a cytochrome P450 named cyp68.4 is the major mechanism responsible for the resistance. This is the first case of a functional validation of fungicide detoxification involved in resistance.

Fungicide use and the emergence of azole resistance in the opportunistic mold *Aspergillus fumigatus.* Paul E. Verweij. Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands

Resistance of fungi to antifungal agents has always been perceived as a minor factor in the outcome of invasive fungal infections. Until recently the species identification of the infecting fungus was an important aid to guide antifungal therapy. However, evidence is accumulating that indicates that acquired resistance may develop and contributes to treatment failure. This has been observed most notably in aspergilli that become resistant to antifungal azoles.
The class of the azoles have become the most prominent class of compounds for the management of invasive aspergillosis. The clinically licensed triazoles with activity against *Aspergillus* include itraconazole, voriconazole and posaconazole. The azoles interact with the biosynthesis of ergosterol, which is an important component of the fungal cell membrane. *Aspergillus* species with acquired resistance to azoles have been reported recently, especially in *A. fumigatus*. It appears that resistance may develop during azole therapy, especially in patients with chronic therapy including patients with chronic disease and aspergilloma. Another route of resistance development may be exposure of *Aspergillus* to azole fungicides that are used in our environment. Patients would then inhale azole-resistant conidia and develop azole-resistant aspergillosis. The consequence of this route of transmission is that azole-resistant disease may occur in patients without previous exposure to azole compounds. Azole resistance is commonly due to mutations in the *Cyp51A*-gene and is associated with different phenotypes. One resistance mechanism (TR/L98H) is highly dominant in the Netherlands, but has also been reported in other European countries including Denmark and Norway. *A. fumigatus* isolates with TR/L98H have also been recovered from the environment. Azole-resistant *A. fumigatus* isolates appear to remain virulent and are capable of causing invasive disease in patients at risk. The efficacy of azole compounds against azole-resistant isolates, with different resistance mechanisms, has been investigated in experimental models of invasive aspergillosis. These indicate that the minimal inhibitory concentration (MIC) has major impact on the efficacy of the azole. Recently a second resistance mechanism has emerged in the Netherlands, following a similar pattern to that of TR/L98H. This indicates that the health risks associated with the use of azole fungicides should be reassessed.

Mechanisms of multiple fungicide resistance in *Botrytis cinerea* populations from vineyards and strawberry fields. Michaela Leroch[1], Andreas Mosbach[1], Cecilia Plesken[1], Dennis Mernke[1], Anne-Sophie Walker[2], Sabine Fillinger[2], Matthias Kretschmer[1] and Matthias Hahn[1]. [1]Department of Biology, University of Kaiserslautern, Postbox 3049, 67663 Kaiserslautern, Germany. [2]INRA-UR 1290 BIOGER-CPP, Avenue Lucien Bretignie`res BP 01, F78850 Thiverval-Grignon, France, E-mail: mleroch@rhrk.uni-kl.de

Botrytis cinerea causes losses of important crops worldwide. Fungicide treatments are effective for plant protection, but bear the risk of resistance development. Monitoring of *Botrytis cinerea* strains in French and German vineyards revealed an increasing occurrence of both MDR (multidrug resistance) strains and specific (target site) resistance against fungicides. Three MDR phenotypes were distinguished according to their fungicide resistance spetra. MDR1 strains show reduced sensitivities against fludioxonil and cyprodinil, and MDR2 strains against fenhexamid and iprodion. MDR3 strains result from recombination of MDR1 and MDR2 strains. In MDR1 strains, several point mutations in a transcription factor encoding gene (*mrr1*) lead to constitutive activation of the ABC transporter gene *atrB*. In MDR2 strains, overexpression of the MFS transporter gene *mfsM2* is a result from two rearrangements in the *mfsM2* promoter caused by insertion of a retroelement derived sequence.
In contrast to 2-3 fungicide treatments against *Botrytis* in vineyards, treatments in strawberry fields often occur weekly, resulting in repeated use of the same fungicides. *Botrytis* strains isolated from German strawberry fields showed a high occurrence of multiple fungicide resistance, due to a combination of specific and MDR resistance mechanisms. We also detected a new MDR phenotype ($MDR1^h$), with higher resistance levels than MDR1 strains, leading to higher constitutive overexpression of *atrB*. Interestingly, most of the strawberry isolates show significant sequence divergence compared to known *B. cinerea* populations from vineyards indicating a novel genetic group. The taxonomic status of these isolates and the mutations leading to $MDR1^h$ is currently under investigation.

Remodeling of the fungal cell wall contributes to Fludioxonil and Ambruticin resistance in the dermatophyte *Trichophyton rubrum*. Nalu Peres[1], Diana Gras[1], Pablo Sanches[1], Antonio Rossi[1], Rolf Prade[2], Nilce Martinez-Rossi [1]. [1]University of Sao Paulo - Brazil, [2]Oklahoma State University - USA. e-mail: nalu@usp.br

Fungal infections have become a health problem worldwide, leading to the need for the development of new efficient antifungal agents. Although dermatophytes do not cause life-threatening diseases, there are reports of deep infections and severe lesions in immunossupressed patients and impairment of living standards of the infected individuals. The antifungal compounds Fludioxonil and Ambruticin present a unique mode of action, interfering with the fungal osmotic signaling pathway. We evaluated the effect of these drugs on *Trichophyton rubrum*, and low doses of the drugs inhibit growth of this dermatophyte, leading to hyphal-tip swelling, rupture of cell wall, and leakage of cell contents. We isolated Ambruticin/Fludioxonil resistant mutants with UV, which showed enhanced conidiation, altered pigmentation, modified vegetative growth rates, and higher sensitivity to osmotic stress. Using the sib-selection approach we isolated two genes encoding a phospholipid transporter and the glucan 1,3- beta glucosidase protein, which conferred resistance to Ambruticin and Fludioxonil to the wild type strain. Since these enzymes are involved in the remodeling of fungal cell wall, we suggest that this process may be an important mechanism contributing to the resistance to both drugs. Financial Support: FAPESP, and CNPq.

Global analysis of the evolution and mechanism of echinocandin resistance in a series of *Candida glabrata* clinical isolates. Sheena D. Singh-Babak & Leah E. Cowen Department of Molecular Genetics, University of Toronto, Ontario, Canada

Candida species are the leading fungal pathogens of humans and *C. glabrata* is now second to *C. albicans* as the most prevalent *Candida* species due to its intrinsic resistance to the most widely used class of antifungals, the azoles. As a result, the newest class of antifungals, the echinocandins, is commonly employed to treat *C. glabrata* infection. My work thus far established that the molecular chaperone Hsp90 plays a role in resistance to the cell wall stress exerted by the echinocandins via the client protein calcineurin in *C. albicans*. Here we present new work that implicates both Hsp90 and calcineurin as regulators that enable survival of cell wall stress exerted by echinocandins in a series of *C. glabrata* isolates that evolved drug resistance in a human host. Genome wide sequencing unveils 45797 single nucleotide variants between the latest clinical isolate and the reference sequence CBS138. Strikingly, only 9 non-synonymous SNVs between the early and late clinical isolates were found. Furthermore, we find a mutation in the echinocandin target FKS2 previously reported to confer resistance in *C. glabrata* clinical isolates. Quantitative RT-PCR experiments revealed that deletion of calcineurin blocks the induction of the resistance determinant FKS2. Thus, our work identifies mutations that accompany the evolution of drug resistance in a human host on a genome-wide scale and suggests a new mechanism of resistance to the echinocandins.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Biobased products, Biofuels, and Bioenergy (Walton/Punt) **Merrill Hall**

Systems Biology Approaches to Understanding Plant Cell Wall Degradation in a Model Filamentous Fungus. N. Louise Glass, Plant and Microbial Biology Department, The University of California, Berkeley, CA 94720-3102

The filamentous fungus *Neurospora crassa* is a model laboratory organism, but in nature is commonly found growing on dead plant material, particularly grasses. Understanding the degradation of plant biomass by filamentous fungi will provide insights and tools for improving cellulosic biofuel production. Using functional genomics resources available for *N. crassa*, which include a near-full genome deletion strain set, tools for expression analysis (whole genome microarrays and RNA-Seq) and cell biological tools, we have undertaken a systemwide analysis of plant cell wall and cellulose degradation. We identified approximately 770 genes that showed expression differences when *N. crassa* was cultured on ground *Miscanthus* stems as a sole carbon source. By interrogating the near full-genome deletion set for strains that either increase or decrease celloloytic activity, we have identified novel regulators and predicted secreted proteins that profoundly affect the ability of *N. crassa* to deconstruct plant cell wall material. We show that *N. crassa* relies on a high-affinity cellodextrin transport system for rapid growth on cellulose. Reconstitution of the *N. crassa* cellodextrin transport system in *Saccharomyces cerevisiae* promotes efficient growth of this yeast on cellodextrins. In simultaneous saccharification and fermentation experiments, the engineered yeast strains more rapidly convert cellulose to ethanol when compared with yeast lacking this system. These results show that the powerful tools available in *N. crassa* allow for a comprehensive system level understanding of plant cell wall degradation mechanisms used by a ubiquitous filamentous fungus.

Improving fungal enzymes for biomass conversion. Goutami Banerjee, Suzana Car, John S. Scott-Craig, Melissa Borrusch, Jonathan D. Walton. Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, Michigan, USA

The cost of enzymes for converting plant biomass materials to fermentable sugars is a major bottleneck in the development of a viable lignocellulosic ethanol industry. Commercial enzyme mixtures currently available, which are mainly from species of *Trichoderma* and *Aspergillus*, are complex and poorly defined. In order to lower the cost of enzymes we need enzyme cocktails of higher specific activity. Our approach to this problem is to use pure enzymes to design synthetic, defined, and optimized mixtures. In this way we learn which enzymes are important for biomass deconstruction and in what optimal proportions. Synthetic mixtures also provide a platform that can be used to find new accessory enzymes and better examples of current enzymes. We have made synthetic mixtures optimized for release of glucose and xylose from alkaline pretreated feedstocks containing more than 16 enzyme components using statistical design of experiment and robotic liquid handling systems. To date, our enzymes have been derived from *Trichoderma reesei*, but novel enzymes that act synergistically with those of *Trichoderma* can be discovered and validated with our system. . The goal of this research is to make enzyme mixtures for biomass applications that have higher specific activity and thus lower cost.

Engineering of Saccharomyces cerevisiae for efficient alcoholic fermentation of plant biomass hydrolysates. Antonius (Ton) J.A. van Maris. Delft University of Technology.

Fuel ethanol production from plant biomass hydrolysates by Saccharomyces cerevisiae is of great economic and environmental significance. Construction of yeast strains that efficiently convert potentially fermentable substrates in plant biomass hydrolysates into ethanol is a major challenge in metabolic engineering.In this presentation synthetic biology strategies that enable fermentation of mixtures of glucose, xylose and arabinose as well as reduction of the by product glycerol, will be discussed.

Comparative genomics of xylose-fermenting fungi to enhance microbial biofuel production. Dana J. Wohlbach[1,2], Alan Kuo[3], Trey K. Sato[2], Katlyn M. Potts[1], Asaf Salamov[3], Kurt M. LaButti[3], Hui Sun[3], Alicia Clum[3], Jasmyn Pangilinan[3], Erika Lindquist[3], Susan Lucas[3], Alla Lapidus[3], Robert Zinkel[2], Kerrie W. Barry[3], Igor V. Grigoriev[3], Audrey P. Gasch[1,2] [1]University of Wisconsin-Madison, Madison, WI, [2]Great Lakes Bioenergy Research Center, Madison, WI, [3]US Department of Energy Joint Genome Institute, Walnut Creek, CA.

Cellulosic biomass is an abundant substrate for biofuel production; however, many microbes cannot natively metabolize pentose sugars within hemicellulose. Although engineered *Saccharomyces cerevisiae* can utilize the pentose xylose, the fermentative capacity pales in comparison to glucose, limiting the economic feasibility of industrial fermentations. To better understand xylose utilization for subsequent microbial engineering, we sequenced the genomes of two xylose-fermenting, beetle-associated fungi: *Spathaspora passalidarum* and *Candida tenuis*. To identify genes involved in xylose metabolism, we applied a comparative genomic approach across fourteen Ascomycete genomes, mapping phenotypes and genotypes onto the fungal phylogeny, and measured genomic expression across five Hemiascomycete species with different xylose consumption phenotypes. Together, this implicated many new genes and processes involved in xylose assimilation. Several of these genes significantly improved *S. cerevisiae* xylose utilization when engineered in this species. This work demonstrates the power of comparative methods in rapidly identifying key genes for biofuel production while reflecting on fungal ecology.

Production of Pravastatin by metabolically engineered *Penicillium chrysogenum* cells. M. Hans, B. Meijrink, P. Klaassen, S. Hage, A.Vollebregt, L. Raamsdonk, R. Lau, W. van Scheppingen, J.M. van der Laan, D. Jacobs, M. van den Berg and Roel Bovenberg. DSM Biotechnology Center, Alexander Fleminglaan 1, 2613AX Delft, The Netherlands

The filamentous fungus *Penicillium chrysogenum* is widely used in industry for the fermentative production of antibiotics such as penicillins and cephalosporins. By employing decades of classical strain improvement, strains were identified which showed increasingly higher productivities as well as beneficial fermentation behaviors. Given the often lengthy and therefore costly strain development programs for new products, it would be desirable to be able to benefit from such improved strain lineages by using them to create high-productivity industrial strains for novel engineering approaches. Such an approach would lead to shorter and therefore less expensive R&D programs. At DSM, work towards that goal focused on the generation of *Penicillium chrysogenum* strains in which "unwanted" metabolites such as beta-lactams were abolished, but still harbored the beneficial features leading to high fermentation productivities. Such "empty strains" were harnessed for the strain development of novel fermentation processes of substances different from beta-lactams and unknown to the *Penicillium chrysogenum* metabolism. As a main example, construction of a high productivity strain for the cholesterol lowering drug Pravastatin is described. Currently, Pravastatin is industrially produced by *Penicillium citrinum* fermentation of the natural product precursor compactin followed by a bioconversion with *Streptomyces carbophilus*, yielding the hydroxylated product pravastatin. DSM's breakthrough technology resulted in a *Penicillium chrysogenum* one-step fermentation ensuring an environmentally and economically advantageous process. Towards this goal, the whole compactin biosynthetic gene cluster from *Penicillium citrinum* was heterologously expressed in *Penicillium chrysogenum*, yielding high productivities of compactin. Subsequently, a P450 compactin hydroxylase was discovered and engineered leading to high levels of pravastatin production. Further strain and process improvements including up-scaling to several cubic meter fermentation volumes were carried out.

Oxido-reductive metabolism of L-arabinose and D-galactose in filamentous fungi: Metabolic crosstalk versus specific enzymes. Dominik Mojzita, Outi M. Koivistoinen, Kiira Vuoristo, Laura Ruohonen, Merja Penttilä and Peter Richard VTT Technical Research Centre of Finland, Espoo, Finland dominik.mojzita@vtt.fi

L-arabinose, the second most abundant pentose sugar, is used as a carbon source by a variety of microorganisms living on decaying plant material. Fungal microorganisms catabolize L-arabinose through an oxido-reductive pathway. We have identified two missing links in the pathway, L-arabinose and L-xylulose reductases in *A.niger*. D-galactose is a relatively rare hexose sugar in the plant cell wall mainly found in galactoglucomannan. There are three pathways indentified in fungi for D-galactose degradation; 1) the Leloir pathway in which D-galactose is phosphorylated, 2) the oxidative pathway which starts by an extracellular galactose oxidase reaction, and 3) a recently proposed oxido-reductive pathway which resembles the pathway for L-arabinose catabolism. It has been suggested in *T. reesei* and *A. nidulans* the oxido-reductive D-galactose pathway employs the enzymes from the L-arabinose pathway. It starts with the conversion of D-galactose to D-galactitol, probably carried by the xylose/arabinose reductase. The second step is catalyzed by L-arabitol dehydrogenase and the product of the reaction is an unusual sugar L-xylo-3-hexulose. We have identified the L-xylulose reductase possesses the activity with this intermediate which is converted to D-sorbitol. Finally, D-sorbitol is oxidized to D-fructose, which enters glycolysis. We have studied the pathway in *A. niger* and uncovered a more complex picture. Apart from showing the possible involvement of the L-arabinose pathway enzymes, we identified two dehydrogenases specifically induced on D-galactose, suggesting that *A. niger* might have specific genes for catabolism of D-galactose rather than using metabolic crosstalk suggested for *T. reesei* and *A. nidulans*.

Biobased Antibiotics From Basidios : Identification and manipulation of the pleuromutilin gene cluster from *Clitopilus passeckerianus.* S. Kilaru, C. Collins, A. Hartley, K. de Mattos-Shipley, P. Hayes, Andy M. Bailey* and Gary D. Foster* School of Biological Sciences, University of Bristol, Bristol, BS8 1UG, UK andy.bailey@bristol.ac.uk or gary.foster@bristol.ac.uk

With bacteria becoming resistant to antibiotics, there is a growing need to find new sources of antibiotics. Our work has focussed on the organism *C. passeckerianus* which produces a natural antibiotic, pleuromutilin. Recently, a derivative of pleuromutilin, retapamulin (developed by GSK) was approved for use in humans. Clinical trials have demonstrated its efficacy against certain Gram-positive bacteria including MRSA. We have developed all the tools to manipulate this important organism, and will present results on transformation, gene manipulation and enhancement, as well as gene isolation and mapping. These tools have allowed us to isolate the pleuromutilin gene cluster. Using the molecular tools we have been able to identify all genes involved, their roles, and perhaps most importantly, the ability to manipulate to elevate levels of antibiotic production and deliberately alter products produced. These results demonstrate that we are able to manipulate and control the *Clitopilus* genome. This provides a molecular toolbox which makes it possible to identify and manipulate individual genes of this fungus, and leading to some major new drugs which are not compromised by antibiotic-resistant strains of bacteria. The results will open up major opportunities for other previously intractable systems and antibiotics in fungi.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Production of dicarboxylic acids by *Aspergillus carbonarius*, the engineering of a novel biochemical cell factory. Niels Bjørn Hansen, Mette Lübeck & Peter Stephensen Lübeck Section for Sustainable Biotechnology; Aalborg University Copenhagen. Lautrupvang 15, 2750 Ballerup, Denmark

The production of dicarboxylic acids by *A. carbonarius* is conducted under a WP involved in the European Commission's 7th framework supported Biorefinery project, SUPRABIO. SUPRABIO handles research, development and demonstration of sustainable production of fuels, chemicals and materials from biomass. For the economically and biological production of building blocks (BBs), the most promising BBs have been identified as four carbon 1,4 diacids; specifically the very high valued chiral acids. These acids have been shown to be fermented by several fungi, however, the yields and the productivity have not been shown to be substantial enough to sustain an industrial unit operation. Recent improvements in metabolic engineering have highlighted genomic modifications that increase the cytosolic flux of four carbon diacids in Yeast. Also, the sequencing of acid tolerant fungi unprotected by patenting restrictions opens up for novel cell factories. These issues and improvements lay the grounds for this WP that focuses on the utilization of C5- and C6 sugar biorefinery streams for the fermentation of high valued compounds. We have found a fungal strain that form the basis organism for the development of a novel cell factory by genomic changes that enhance production of defined compounds. Currently progress involves genomic manipulation, biorefinery side stream adaptation, and characterization of genetic changes in *A. carbonarius*.

High Throughput Methods for Filamentous Fungi (Berg-van-den/Wiest) **Heather**

New methods for High Throughput generation of precise gene knock-outs of *Penicillium chrysogenum* . Bianca Gielesen, Linda van den Hoogen, Hilde Huininga, Hesselien Touw, Laurens Ekkelkamp, Richard Kerkman & Marco van den Berg. DSM Biotechnology Center 699-0310, Alexander Fleminglaan 1, 2613 AX Delft, The Netherlands

In recent years the genome sequences of several industrially important filamentous fungi have been deciphered (i.e. *Aspergillus niger, A. oryzae, A. terreus, Hypocrea jecorina* and *Penicillium chrysogenum*). In order to efficiently sort out the functions of all genes in relation to the industrial applications improved methods needed to be developped. Generally, such studies start by detailed gene annotation and genome wide omics studies, using platforms like microarrays, proteomics and *in silico* modelling. However, as a large proportion of the genes are annotated as hypothetical genes, functional gene studies are needed to get insight in a possible correlation between gene and productivity.

Therefore, we set out to significantly improve the available toolbox for precise engineering of filamentous fungi with a special focus on automation and high-throughput experimentation. The tools developped are applicable to all fungi, but here we will report on the application to *P. chrysogenum*. Historically, DSM has a long experience in penicillinG classical strain improvement and has optimized it during the second half of 20th century towards its current efficiency. Several of the rationales learned during those years were applied in order to develop efficient and automated methods. Recently, we reported on the genome sequence of the international laboratory strain Wisconsin54-1255, detailed annotation and transcriptome studies*. Here, we will report the development of new methods which facilitated us to generate precise gene knock-outs of Penicillium chrysogenum in a high throughput setting.

*Van den Berg *et al.* (2008) Genome sequencing and analysis of the filamentous fungus *Penicillium chrysogenum*. *Nat Biotechnol.* **26**:1161-1168.

Fungal enzymes for biomass deconstruction. Suzana Car, Goutami Banerjee, John S. Scott-Craig, Melissa S. Borrusch, Jonathan D. Walton Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, Michigan, USA.

Enzymes for biomass deconstruction are a major cost in the production of ethanol from lignocellulosic biomass. Currently available commercial enzyme cocktails have generally been optimized for acid-pretreated stover from corn and other grasses and are therefore unlikely to be well-adapted for all of the pretreatment/biomass combinations that exist now and that will emerge in the future. In order to understand better which enzymes, and in what proportions, are optimal for biomass deconstruction, we have developed a high-throughput analysis platform called the GLBRC Enzyme Platform (GENPLAT). We have used this platform to optimize synthetic enzyme cocktails on a variety of pretreated feedstocks, and to evaluate more than 18 fungal enzymes in order to understand their roles in biomass deconstruction. To date, all of our enzymes are derived from *Trichoderma reesei*, but many other fungi contain enzymes that might contribute to biomass degradation. We are using GENPLAT and our synthetic mixtures to “bioprospect” for novel accessory enzymes and superior key enzymes. This poster summarizes our effort to better understand the immense potential and roles of fungal enzymes in deconstructing lignocellulosic biomass, with the ultimate goal of contributing to the development of a practical lignocellulosic ethanol industry.

From one to ten thousand mutants: the development of high-throughput methods at the Fungal Genetics Stock Center. Kevin McCluskey. University of Missouri- Kansas City.

Complementing classical forward genetic methods, reverse genetic approaches have added to the wealth of information generated by studying mutant fungal strains. The FGSC originally dealt in individual mutant strains, and approximately 1500 genes were defined by forward genetic approaches. The advent of whole genome sequencing revealed closer to 10,000 genes in this, and other filamentous fungi. To study these genes, a number of groups have developed systematic gene deletion programs. With over 10,000 Neurospora mutants, 2,000 Candida mutants and 1,400 Cryptococcus mutants, the FGSC has leveraged its experience in handling and distributing genetically characterized strains to increase 100 fold the number of strains distributed annually. While techniques for manipulating the yeasts are available, we have developed protocols for arraying and replicating arrayed sets of filamentous fungi and have applied these techniques to produce both standard and custom arrayed mutant sets. These arrayed sets of Neurospora deletion mutants have been distributed around the world and are contributing to the growth in labs using Neurospora as a model filamentous fungus. Complementary research at the FGSC has led to the development of a novel selectable marker as well as characterization multiple classical mutants by whole genome sequencing.

Comparative phenotyping coupled with high throughput forward genetics and gene deletion strategies reveals novel determinants of pathogenicity in the rice blast fungus *Magnaporthe oryzae*. Janet Wright, Cristian Quispe Jessie Fernandez, David Hartline, Karina Stott, Anya Seng, Jonathan Hinz and Richard A. Wilson. Department of Plant Pathology, University of Nebraska-Lincoln, USA. rwilson10@unl.edu .

To cause rice blast disease, *Magnaporthe oryzae* has distinct morphogenetic stages that allow it to breach the surface of the host leaf and invade the plant tissue. The sugar sensor trehalose-6-phosphate synthase (Tps1) monitors the transition from the nutrient-free surface to the nutrient-rich interior of the leaf and regulates plant infection via a NADP(H)-dependent genetic switch. However, which metabolic and regulatory pathways are required for the fungus to adapt to the fluctuating nutritional environment of the plant host, and how it acquires nutrient during its biotrophic growth phase, is not known. Therefore, using simple plate tests, we sought to determined which biochemical pathways, over- or under-represented in the plant pathogen *M. oryzae* compared to the soil saprophyte *Aspergillus nidulans*, could be required for the rice blast lifestyle. We also compared to wild type the metabolic diversity of key *M. oryzae* regulatory mutants, such as Δtps1 and Δnut1 deletion strains (the latter required for nitrogen source utilization). Finally, we coupled this comparative phenotyping study to high throughput Agrobacterium-mediated forward genetics and gene deletion strategies to rapidly identify and functionally characterize the role of important biochemical and regulatory pathways in disease establishment. In this manner, we report here how carbon catabolite repression and citrate efflux is essential for virulence, and how perturbing histone gene regulation results in severe condial reduction and complete loss of pathogenicity in the devastating rice blast fungus.

A reverse and forward genetic clock-screening strategy to identify new circadian regulators in *Neurospora crassa*. Luis F. Larrondo^ Jennifer J. Loros*, Jay C. Dunlap* and Alejandro Montenegro-Montero^ ^- DGMM. P. Universidad Católica de Chile. *- Dept. Genetics, Dartmouth Medical School, USA.

Neurospora circadian rhythms can be indirectly followed by the overt rhythmic appearance of spores (conidial banding). Mutations that affect circadian-gene expression, but not overt rhythmic conidiation, are normally overlooked. To overcome this and other limitations a fully-codon optimized luciferase reporter system for *N. crassa* was developed. By putting this real-time reporter under the control of promoter regions containing circadian elements, rhythms in transcription of *frq* (oscillator component) or clock-controlled genes (*ccgs*) can be easily tracked for over a week. Moreover, by generating FRQ-LUC translational fusion strains, rhythms in FRQ protein can be followed in a semi- quantitative manner. By using a bioluminescence high-throughput screening platform and following a reverse and forward genetic screening strategy and functional genomic tools, we have started to identify interesting candidates affecting either the core oscillator or the output pathways. Thus, we have identified at least one transcription factor that regulates the expression of some *ccgs*, potentially representing a direct link between the WCC (core oscillator) and the downstream output machinery. In addition, we have started to map a new mutant displaying both a period defect and female sterility. As a result, this new experimental setup has started to reveal novel molecular details of the Neurospora clock. Funding: FONDECYT 1090513

Screens of a *Candida albicans* homozygous gene disruption library reveal novel regulators of virulence and commensalism Suzanne Noble MD, PhD. Department of Medicine, Division of Infectious Diseases and Department of Microbiology & Immunology, UCSF School of Medicine, San Francisco, CA

Candida albicans is a normal commensal of the human gut microbiome, as well as the most common cause of disseminated fungal infections. Its ability to transition among yeast, hyphal, and pseudohyphal morphologies has been strongly linked to virulence. We constructed a library of nearly 700 homozygous gene knockout mutants of this obligate diploid organism and screened the mutants for competitive fitness in a mouse model of disseminated disease, as well as for morphogenesis and proliferation rate *in vitro* (Noble et al., 2010). Our results confirmed a strong association between morphogenetic transitions and virulence but also highlighted a group of mutants that affect fitness in the host independently of morphogenesis or proliferation rate. Focused studies of the virulence-specific mutants have exposed several novel pathways of virulence, including an iron homeostasis regulatory pathway that we demonstrate is also required for the commensal lifestyle of this ubiquitous mammalian fungus.

Noble, S.M., French, S., Kohn, L.A., Chen, V., and Johnson, A.D. (2010). Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat Genet *42*, 590-598.

High throughput analysis of gene function by comparative genomics. Masayuki Machida[1], Hideaki Koike[1], Yoshinori Koyama, Myco Umemura[1*], Hiroko Hagiwara[1], Tomoko Ishii[1], Tomomi Toda[1], Noriko Yamane, Akira Ohyama[2], Jiujiang Yu[3], Thomas E. Cleveland[3], Keietsu Abe[4], Motoaki Sano[5], Shinichi Ohashi[5], Tsutomu Ikegami[1], Makoto Yui[1], Yusuke Tanimura[1], Isao Kojima[1], Satoshi Sekiguchi[1], Goro Terai[6], Toshitaka Kumagai[7], Toutai Mitsuyama[1], Katsuhisa Horimoto[1], Kiyoshi Asai[1] [1]Natl. Inst. Adv. Ind. Sci. Tech. (AIST), [2]Insilico Biology, [3]South Reg. Res. Center (SRRC), [4]Tohoku U., [5]Kanazawa Inst. Tech. (KIT), [6]INTEC Systems Inst., [7]Fermlab.

Comparative genomics and comparative functional genomics are known to be a powerful tool to address gene function. We have successfully applied it to predict candidates of essential genes from Aspergillus oryzae for the screening of anti-fungal reagents. It also provides useful information for exploring secondary metabolism genes. Currently, we are sequencing several genomes of microorganisms with unique characteristics including yeasts and filamentous fungi, and are analyzing their transcription expression profiles by DNA microarray and metabolites by LC/MS. The information generated is subjected to our bioinformatics pipelines including assembling, annotation and other analyses by the tools developed by our informatics team. Since general methods in the comparative genomics fields have not been well established, we are developing add-on tools to incorporate new features for the software that flexibly displays sequences, genes, annotations, expressions from DNA microarray. By this software, any additional information of interest can be superimposed onto the genetic information by importing the data in various formats such as GenBank, GFF and so on. Examples of our trial by using the platform above will be discussed.

Secretome discovery reveals lignocellulose degradation capacity of the ectomycorrhizal fungus *Paxillus involutus*. Doris Roth[1], François Rineau[2], Peter B. Olsen[3], Tomas Johansson[2], Andrea L. L. Vala[1], Morten N. Grell[1], Anders Tunlid[2], Lene Lange[1]. [1]Section for Sustainable Biotechnology, Aalborg University, Copenhagen, Danmark. [2]Department of Microbial Ecology, Lund University, Sweden. [3]Novozymes A/S, Bagsværd, Danmark. droth@bio.aau.dk

To improve our understanding of the role ectomycorrhizal fungi play in biomass conversion, we studied the transcriptome of *P. involutus* grown on glass beads in extract of soil organic matter. The mycelium was used for a cDNA library screened by Transposon-Assisted Signal Trapping (TAST[*]) for genes encoding secreted proteins. We identified 11 glycoside hydrolases (GH), none of them being cellulases of the GH families 6, 7 and 45, which constitute the well described enzymatic cellulose degradation system from numerous efficient cellulolytic fungi. In contrast, several predicted enzymes, namely a laccase and oxidoreductases possibly contribute to hydroxyl radical formation. The most abundant GH found was GH61, although typically described as accessory protein in the enzymatic cellulolytic apparatus. All in all, our results suggest that the cellulose degradation system of *P. involutus* resembles the brown rot fungi systems. In addition, GH61 apparently acts as accessory protein both in enzymatic and in radical-based cellulolysis. [*] Becker et al., J. Microbial Methods, 2004, 57(1), 123-33

Population Genomics (Anderson/Stukenbrock) **Kiln**

Evolution of lineage-specific chromosomes in the *Fusarium oxysporum* species complex Sarah M. Schmidt[1], Li-Jun Ma[2,3], H. Corby Kistler[4] and Martijn Rep[1] [1] Plant Pathology, SILS, University of Amsterdam, 1090 GE Amsterdam, The Netherlands [2] Broad Institute, Cambridge, MA, [3] University of Massachusetts Amherst, MA, USA, [4] Plant Pathology, ANRS, 6030 St Paul Campus, MN, USA.

Fusarium oxysporum is a soilborne fungus that causes wilt disease in many plant species by colonizing the host xylem vessels. The *F. oxysporum* species complex is a collection of apparently asexual non-pathogenic and pathogenic clonal lineages. Many lineages harbor unique genomic sequences residing mostly on extra chromosomes. In the tomato wilt strain *F. oxysporum* f. sp. *lycopersici* (*Fol*) the phylogenetic history of these lineage- specific (LS) chromosomes differs from the core genome. To investigate the origin of the LS chromosomes and their relation to host specificity within the *F. oxysporum* species complex we are currently analyzing genomic sequences of strains with host specificities towards Arabidopsis, cotton, human, pea, banana, cabbage, radish and melon. This genomics approach is complemented by a proteomics approach. In the interaction between *Fol* and tomato, the fungal effectors that are secreted in the xylem sap are crucial determinants of virulence and are encoded on LS chromosomes. We are presently investigating *Fo* f. sp. *melonis* effectors that are secreted during melon infection, as a first step towards assigning a virulence function to LS genes in this pathogenic strain.

Sexual recombination and the possibility of cryptic heterokaryosis in *Aspergillus flavus*. Rodrigo A. Olarte[1], Bruce W. Horn[2], James T. Monacell[3], Rakhi Singh[1], Eric A. Stone[3,4], Ignazio Carbone[1]. [1]Plant Pathology, NCSU, Raleigh, NC 27695 [2]NPRL, USDA-ARS, Dawson, GA 39842 [3]BRC, NCSU, Raleigh, NC 27695 [4]Genetics, NCSU, Raleigh, NC 27695

Aspergillus flavus infects both plants and animals and is of toxicological importance due to its production of aflatoxins (AFs). Recent efforts to reduce AF concentrations have focused on the use of the biocontrols AF36 and Afla- Guard®, both of which contain nonaflatoxigenic *A. flavus* strains as an active ingredient. Biocontrols are applied to fields, where they competitively exclude native aflatoxigenic strains. Although biocontrol is effective in reducing AF contamination in crops, the extent to which these strains recombine with native strains and the overall effect on fungal populations are unknown. Here we show that the recombination breakpoints in the F1 correlate with the breakpoints inferred from population genetic studies of natural isolates. Furthermore, we demonstrate that a crossover event within the AF cluster can repair a nonsense mutation, resulting in a regained aflatoxin-producing phenotype. Finally, we observed non-Mendelian inheritance of extra-genomic AF cluster alleles in crosses with partial AF cluster parents, suggesting a possible role of cryptic heterokaryosis, in addition to sexual recombination, in modulating AF production. Collectively, these processes may contribute to increased effective population sizes and drive genetic and functional hyperdiversity in *A. flavus*.

Population genomics and local adaptation in *Neurospora crassa* isolates from the Caribbean Basin. Christopher E. Ellison[1], Charles Hall[1], David Kowbel[1], Juliet W. Welch[1], Rachel B. Brem[2], N. Louise Glass[1], John W. Taylor[1] Departments of [1]Plant & Microbial Biology and [2]Molecular and Cell Biology, University of California, Berkeley, CA 94720-3102, USA. cellison@berkeley.edu

The elucidation of the genetic basis of adaptation is a highly sought after, yet rarely achieved goal. Thus far, most instances where adaptive alleles have been discovered involved identifying candidate genes based on their having a function related to an obvious phenotype such as pigmentation. This "forward-ecology" approach is difficult for most fungi because they lack obvious phenotypes. We have used a "reverse-ecology" approach to identify candidate genes involved in local adaptation to cold temperature in two recently diverged populations of *Neurospora crassa* by performing high-resolution genome scans between populations to identify genomic "islands" of extreme divergence. We find two such islands containing genes whose functions, pattern of nucleotide polymorphism, and null phenotype are consistent with local adaptation.

The making of a new pathogen: Insights from comparative population genomics of the domesticated wheat pathogen *Mycosphaerella graminicola* and its wild sister species. Eva H. Stukenbrock[1)], Thomas Bataillon[2)], Julien Y. Dutheil[2)], Troels T. Hansen[2)], Ruiqiang Li[3)], Marcello Zala[4)], Bruce A. McDonald[4)], Wang Jun[3,5)], Mikkel H. Schierup[2)] . [1)]Max Planck Institute for Terrestrial Microbiology, Karl von Frisch Str., D-35043 Marburg, Germany, [2)]Bioinformatics Research Center, Aarhus University, C.F. Moellers Alle, Bldg 1110, DK-8000 Aarhus C, Denmark, [3)] Beijing Genomics Institute, Shenzhen 518083, China, [4)]ETH Zurich, Inst. Integrative Biology, Universitätsstrasse 2, 9082 Zurich, Switzerland, and [5)]Dept Biology, University of Copenhagen, Ole Maaløs vej 5, DK-2200 Copenhagen, Denmark.

The fungus *Mycospherella graminicola* emerged as a new pathogen of cultivated wheat during its domestication about 11.000 years ago. We assembled 12 high quality full fungal genome sequences to investigate the genetic footprints of selection in the pathogens and closely related sister species that infect wild grasses.
Positive selection and adaptive evolution have prominently altered genes encoding secreted proteins and putative pathogen effectors supporting the premise that molecular host-pathogen interaction is a strong driver of pathogen evolution.
We demonstrate a strong affect of natural selection in shaping the pathogen genomes. Adaptive evolution has to a higher extent affected *M. graminicola* consistent with recent host specialization. The strong impact of natural selection, we document, is at odds with the small effective population sizes estimated and suggests that population sizes fluctuate significantly.
Recent divergence between pathogen sister species is manifested in the high degree of incomplete lineage sorting (ILS) in their genomes. We exploit ILS to generate a genetic map of the species and document recent times of species divergence relative to genome divergence. We show highly different evolutionary patterns in the "domesticated" pathogen compared to its wild relatives suggesting that emergence of a new agricultural host selected a highly specialized and fast evolving pathogen.

Sex determination in the original sexual fungus. Alexander Idnurm. School of Biological Sciences, University of Missouri-Kansas City, 5100 Rockhill Road, Kansas City MO 64110, USA

The first report of sex in the fungi dates two centuries ago to the species *Syzygites megalocarpus* (Mucormycotina). This organism was used by Blakeslee as a representative of self-fertile species, leading to the development of the concepts of heterothallism and homothallism for the kingdom. Here, two putative *sex*/*MAT* loci were identified in a single strain of *S. megalocarpus*, revealing the basis for homothallism. The species encodes copies of both of the HMG-domain containing SexM and SexP proteins, flanking by conserved RNA helicase and glutathione oxidoreductase genes found adjacent to the mating type loci in other Mucormycotina species. The presence of pseudogenes and the arrangement of genes suggests the origin of homothallism in this species from a heterothallic relative via chromosomal rearrangements to bring together both loci into a single genetic background.

Population genomics of the amphibian pathogen *Batrachochytrium dendrobatidis* from genome resequencing. Jason E Stajich[1], Suzanne Joneson [2], Tim Y James[3], Kelly Zamudio[4], Erica Bree Rosenblum[2]. [1]University of California, Riverside, CA. [2]University of Idaho, Moscow, ID. [3]University of Michigan, Ann Arbor, MI. [4]Cornell University, Ithaca, NY. jason.stajich@ucr.edu

Bd is an emerging infectious disease linked to worldwide amphibian decline. Global genotypic variation appears to be low, but some private geographic-specific alleles are present suggesting genetic isolation of strains. Using the whole genome sequencing of strains JAM81 (Joint Genome Institute) and JEL423 (Broad Institute) as references we re-sequenced and identified polymorphisms in an additional 24 strains to compare variation among isolates from the Western & Eastern US and Central & South America. The Bd genome is diploid and previous work (James et al, 2009) has identified regions of loss of heterozygosity (LOH) from Multi Locus Sequencing markers. Analysis of whole genomes of multiple strains now identifies precise genomic locations of independent and shared LOH events. We have found that strains from Central and South America have lost alleles that are observed in North America due to reciprocal crossover of chromosome arms. The resequencing data from geographically and genetically diverse strains allow us to build a high-resolution inventory of genetic variation useful for future developments of high throughput sample genotyping and tracing origins of Bd outbreaks.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

How Polyploidy and Aneuploidy Impact the Speed of Adaptation. Anna Selmecki, Marie Guillet, Noam Shoresh, Roy Kishony, David Pellman. Dana-Farber Cancer Institute and Harvard Medical School

Variation in chromosome content, either through duplications of whole chromosome sets (polyploidy) or by alterations in chromosome number (aneuploidy), are observed during development, can promote tumorigenesis, and in microorganisms they can be linked to specific evolutionary adaptations. An extensive body of theory suggests ways in which polyploidy might accelerate evolution, however, there has been a paucity of quantitative experimental tests of these ideas. Here we report a quantitative study of mutation, selection, and fitness in yeast strains engineered to have increased ploidy. *In vitro* evolution experiments were performed to determine the beneficial mutation rate and fitness benefit of these mutations in populations of isogenic strains. We have used a population dynamics model developed by our collaborators[1] to quantitatively measure the effect of polyploidy on the rate and dynamics of adaptation to raffinose. We identified specific mutations that haploid (1N), diploid (2N), and tetraploid (4N) yeast acquired during adaptation with microarray comparative genome hybridization, expression array analysis, and whole genome resequencing. We then characterized the fitness effects of these mutations relative to the ancestor strains. Our results indicate that during evolution in raffinose beneficial mutations arise faster in the 4N competitions compared to the 1N or 2N competitions, and 4N populations take more adaptive steps in the same number of generations as 1N and 2N populations. Whole chromosome and segmental aneuploidy occurs frequently in the evolved 4N clones, but was never detected in the 1N or 2N populations. We hypothesize that the increased evolvability seen in 4N strains may be mediated, at least in part, by chromosome instability. We generated mutants to test this hypothesis directly and their evolutionary outcomes will be discussed. Together our approach provides the first quantitative experimental characterization of how polyploidy and aneuploidy alter the rate of adaptation.

1. Hegreness, M., N. Shoresh, D. Hartl, and R. Kishony. 2006. An equivalence principle for the incorporation of favorable mutations in asexual populations. Science. 311:1615-1617.

Host induced epigenetic alteration in *Phytophthora ramorum*. Takao Kasuga[1], Melina Kozanitas[2], Mai Bui[1], Daniel Hüberli[3], David Rizzo[4] and Matteo Garbelotto[2]. [1]USDA-ARS, Davis, CA, USA, [2]UC Berkeley, CA, USA, [3]DAF, South Perth, Australia, [4]UC Davis, CA, USA

An oomycete plant pathogen *Phytophthora ramorum* is responsible for two distinctive diseases; (1) Sudden Oak Death, which is characterized by lethal bole cankers on oaks, and (2) Ramorum blight, which causes necrotic lesions on leaves of diverse shrub species such as bay laurel and Rhododendrons (foliar hosts). It has been noticed that although a single clonal lineage dominates in Californian forests, isolates originating from oaks tend to be less virulent on both oak and foliar hosts than those from foliar hosts, and colonies of oak isolates look irregular and are somatically unstable. We hypothesized that because *P. ramorum* in California is exclusively clonal, most of the aforementioned phenotypic variations should be due to difference in gene regulation rather than genetic polymorphism. We have conducted microarray mRNA profiling and found that hundreds of genes encoding for transposable elements were highly active in some isolates derived from oak trees, which we termed transposon derepressed phenotype (TDP). RT-qPCR was then employed to measure the expression level of transposons in one hundred *P. ramorum* isolates derived from diverse host species. It was found that 64% of isolates derived from oak hosts showed TDP, whereas only 9% of isolates from foliar hosts showed TDP. We hypothesize that *P. ramorum* incurs epigenetic alterations within and beneath oak bark, which resulted in derepression of transposons.

Septin-mediated morphological transitions during plant infection by the rice blast fungus. Yasin F Dagdas and Nicholas J Talbot Biosciences, College of Life and Environmental Sciences, Geoffrey Pope, University of Exeter, Stocker Road, Exeter, EX4 4QD, UK

Magnaporthe oryzae is the causal agent of rice blast disease, which is a serious threat to global food security. Global yield losses caused by the fungus are approximately $6 billion per annum. *M. oryzae* undergoes several morphogenetic transitions during plant infection and tissue colonization and this plasticity is important for pathogenicity. However, it is not known how cell shape is controlled during the infection-associated developmental phases exhibited by the fungus. Septins are small GTPases that are cytoskeletal elements known to control various morphogenetic events in both yeasts and filamentous fungi. We reasoned that septins might be important regulators of infection-related development in Magnaporthe. We generated an isogenic set of five mutants, each differing by a single septin gene. We also constructed strains of *M. oryzae* expressing fluorescently-labelled septins to facilitate live cell imaging of septin hetero- oligomeric complexes during plant infection. We observed that all septin mutants are defective in pathogenesis and exhibit abnormal cell shapes. Septins form a wide variety of structures, including collars, rings, filaments, bars and patches. The *sep3* mutant is completely non-pathogenic and also appears to be defective in cell cycle progression, the cell integrity pathway and actomyosin ring formation. We also speculate that septins may act as diffusion barriers during appressorium development, based on abnormal localisation of appressorium-specific gene products in septin-deficient mutants. An investigation into the role of septins during plant pathogenesis will be presented.

***RIG1*, a gene essential for pathogenicity in *Magnaporthe oryzae*, is representative of Gti1_Pac2 family members required for invasive growth in fungal pathogens of plants and animals.** Amritha S. Wickramage, M. Alejandra Mandel and Marc J. Orbach, Department of Plant Sciences, Division of Plant Pathology and Microbiology, University of Arizona, Tucson, AZ 85721.

Magnaporthe oryzae has two members of the fungal gene family Gti1_Pac2 – a group of genes that regulate phase transition in human pathogens *Candida albicans (WOR1), Histoplasma capsulatum (RYP1)* and the plant pathogen *Fusarium oxysporum (SGE1)*. Deletion mutants generated separately for each gene showed that one member - *RIG1 (Required for Infectious Growth 1)* - but not the other, MGG_06564, was important for pathogenicity. RIG1 is dispensable for vegetative growth, but *RIG1* deletion mutants (*rig1*) are non-pathogenic, even after removal of the penetration barrier. Microscopic analysis of the mutant from germination through infection indicate that the mutant forms significantly longer germ tubes than the wildtype parental strain 70-15, but forms appressoria that are morphologically and functionally identical to those of 70-15. Observation of fluorescent protein- tagged strains indicates that the mutant fails to form primary infectious hyphae *in planta*: the point synonymous to phase transition in animal pathogenic fungi. RIG1 transcript levels are upregulated in mature appressoria of the wild type, relative to the mycelium. Based on the observed function of *RIG1* homologs in the Gti1_Pac2 family, we propose that these members represent a consensus gene required for invasive growth within the host in both animal- and plant-pathogenic fungi.

Hyphal development in *Candida albicans* requires two temporally linked changes in promoter chromatin for initiation and maintenance. Yang Lu, Chang Su, Allen Wang and Haoping Liu. Department of Biological Chemistry, University of California, Irvine, CA 92697, USA.

Phenotypic plasticity is common in development. For *Candida albicans*, the most common cause of invasive fungal infections in humans, morphological plasticity is its defining feature and is critical for its pathogenesis. Unlike other fungal pathogens that exist primarily in either yeast or hyphal forms, *C. albicans* is able to switch reversibly between yeast and hyphal growth forms in response to environmental cues. Although many regulators have been found involved in hyphal development, the mechanisms of regulating hyphal development and plasticity of dimorphism remain unclear. Here we show that hyphal development involves two sequential regulations of the promoter chromatin of hypha-specific genes. Initiation requires a rapid but temporary disappearance of the Nrg1 transcriptional repressor of hyphal morphogenesis via activation of the cAMP-PKA pathway. Maintenance requires promoter recruitment of Hda1 histone deacetylase under reduced Tor1 (target of rapamycin) signalling. Hda1 deacetylates a subunit of the NuA4 histone acetyltransferase module, leading to eviction of NuA4 acetyltransferase module and blockage of Nrg1 access to promoters of hypha-specific genes. Promoter recruitment of Hda1 for hyphal maintenance happens only during the period when Nrg1 is gone. The sequential regulation of hyphal development by the activation of the cAMP-PKA pathway and reduced Tor1 signalling provides a molecular mechanism for plasticity of dimorphism and how *C. albicans* adapts to the varied host environments in pathogenesis. Such temporally linked regulation of promoter chromatin by different signalling pathways provides a unique mechanism for integrating multiple signals during development and cell fate specification.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Characterization of ZNF2 as a master regulator for hyphal morphogenesis and virulence in *Cryptococcus neoformans.* Linqi Wang and Xiaorong Lin. Texas A&M University, College Station, TX, xlin@mail.bio.tamu.edu

Dimorphism is a common feature that is usually associated with virulence potential in many dimorphic fungal pathogens. However, this association remains elusive in *Cryptococcus neoformans*, a major causative agent of fungal meningitis, in which filamentation is usually observed during mating. This is partially due to limited knowledge of filamentation-specific determinants in *C. neoformans*. We previously revealed that Znf2 is a terminal regulator for hyphal morphogenesis. Deletion of Znf2 completely abolishes filamentation and increases virulence in the animal model of cryptococcosis, suggesting that Znf2 plays a pivotal role in linking cryptococcal dimorphism and virulence. To further address the role of Znf2 in hyphal morphogenesis, we overexpressed Znf2 in the wild-type (JEC21|Á), as well as *mf|Á1,2,3|¤* and *mat2|¤* mutants in which the pheromone sensing pathway is blocked and self-filamentation is almost abolished. Overexpression of Znf2 in all backgrounds leads to extremely robust self-filamentation, indicating the role of Znf2 as a master regulator in cryptococcal self-filamentation. The effect of Znf2 overexpression on self- filamentation was found to be independent of serotype or mating type. Further dissection of roles of Znf2 in hyphal production and virulence is expected to provide not only the critical link for cryptococcal dimorphism and virulence but also a general mechanism underlying dimorphism and virulence among evolutionarily diverse fungal species.

The calcineurin pathway governs dimorphic transition in the pathogenic zygomycete *Mucor circinelloides.* Soo Chan Lee, Cecelia Shertz, Robert Bastidas, and Joseph Heitman Department of Molecular Genetics and Microbiology, Duke University, Durham, NC.

The calcineurin pathway is conserved from yeast to humans and controls numerous cellular processes. In pathogenic fungi, calcineurin functions in both growth and pathogenesis, which makes calcineurin inhibitors attractive antifungal drug candidates. Interestingly, FK506 drives *Mucor circinelloides* to grow as yeast, which is also observed in anaerobic and high CO_2 growth conditions. *M. circinelloides* has three calcineurin subunit A's (CnaA, CnaB, and CnaC) and one calcineurin B subunit (CnbA). CnaC is highly expressed during anaerobic or FK506 treatment, in which the fungus grows as a yeast. *M. circinelloides* grows filamentously in the absence of FK506 and in aerobic conditions, where the expression of CnaC is lower. The expression of CnaA and CnaB were not altered during the dimorphic switch. Interestingly, an FK506-resistant mutant expressed higher levels of CnaC in the presence of FK506 and displayed hyphal growth. *Rhizopus oryzae* also has three calcineurin A subunit genes with a single calcineurin B subunit. Further analysis revealed that three copies of *cna* might have involved a whole genome duplication in *R. oryzae* and individual gene duplications in *M. circinelloides*. *Phycomyces blakesleeanus* has a single copy of gene for each subunit. Our results demonstrate that the calcineurin pathway regulates dimorphic transition in *M. circinelloides* and variation in the evolutionary trajectory of the calcineurin pathway has been adapted in zygomycetes.

Shared regulation during asexual development and dimorphic switching in the human fungal pathogen *Penicillium marneffei*. Tan, K, Bugeja, H. E., Canvas, D., Boyce, Kylie. J. and Andrianopoulos, Alex . Department of Genetics, University of Melbourne, 3010, AUSTRALIA.

Penicillium marneffei is an emerging fungal pathogen of humans, in particular those who are immunocompromised. *P. marneffei* has the capacity to alternate between a hyphal and a yeast growth form, a process known as dimorphic switching, in response to temperature. *P. marneffei* grows in the hyphal form at 25˚C and in the yeast form at 37˚C. The hyphal form produces conidia which are likely to be the infectious agent while the yeast growth form is the pathogenic form found in infected patients. These yeast cells exist intracellularly in the mononuclear phagocyte system of the host. The molecular events which establish and maintain the developmental states and control of the dimorphic switching process in *P. marneffei* are poorly understood.
The *abaA* gene is a member of the ATTS class of transcriptional regulators which control developmental processes in eukaryotes. In *P. marneffei* and *Aspergillus nidulans, abaA* is a key transcriptional regulator of asexual development (conidiation) and in particular phialide differentiation. In addition, *P. marneffei abaA* controls yeast cell morphogenesis during the dimorphic switch, and mutants produce aberrant multinucleate yeast cells. In *Saccharomyces cerevisiae* and *Candida albicans*, the *abaA* homologue *TEC1* regulates filamentation during pseudohyphal growth and hyphal morphogenesis, respectively. The regulatory pathway in which *abaA* operates is very well characterised for conidiation in *A. nidulans*. An extensive analysis of the upstream and downstream factors of this pathway has been conducted to understand which elements of this pathway are shared during conidiation and dimorphic switching. In addition, an examination of the regulatory signals that control *abaA* expression during conidiation and dimorphic switching has been performed. The data show that the *brlA* gene encodes a C_2H_2 zinc finger transcriptional regulator which is known to regulate *abaA* expression does not control dimorphic switching. Instead, promoter analysis has defined a newly evolved region which regulates *abaA* expression during yeast cell morphogenesis.

Morphological heterogeneity *of Paracoccidioides brasiliensis*: characterization and relevance of the Rho-like GTPase *Pbcdc42*
Menino J[1], Barros D[1], Gomes-Alves AG[1], Hernández O[2,3], Almeida AJ[1], Fernando Rodrigues[1]. [1]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal, [2]Instituto de Biología, Universidad de Antioquia, Medellín, Colombia, [3]Cellular and Molecular Biology Unit, Corporación para Investigaciones Biológicas (CIB)

Paracoccidioides brasiliensis is the etiological agent of paracoccidioidomycosis, a disease majorly confined to Latin America and of evident significance in the endemic areas. This fungus is characterized by its ability to change from a mycelial non-pathogenic form at environmental temperatures (22-25ºC) to a pathogenic multiple-budding yeast form at host temperatures (37ºC). In addition to its unique budding pattern and cell division, *P. brasiliensis* yeast cells are also characterized by a polymorphic growth, leading to the formation of both mother and bud cells with extreme variations in shape and size. We have previously shown the involvement of Pbcdc42p in the cell size of *P. brasiliensis* using anti-sense RNA technology targeting this molecule. Further studies have been performed to characterize different *P. brasiliensis* isolates, clinical and environmental, from the 3 different lineages (S1, PS2, and PS3) in terms of both morphological heterogeneity of buds and mother cells and the expression of *PbCDC42*. Our results reveal high heterogeneity both within the same isolate and amongst different isolates regarding cell size and shape of the mother and bud cells. Bud number per mother cell was also highly variable, suggesting at least a distinct regulation of the budding pattern. No associations were detected between mother cell and bud cell size and shape. On the other hand, our data seems to point out a correlation between *PbCDC42* expression and some of the previously mentioned morphological characteristics. Altogether, this study provides a quantitative evaluation morphological parameters of *P. brasiliensis* yeast cells, reinforcing that *P. brasiliensis* does not follow standard rules of cell division during growth. This work was supported by a grant from FCT – Fundação para a Ciência e a Tecnologia, Portugal (PTDC/BIA-MIC/108309/2008). J.M. and GAAG are recipients of FCT fellowships.

Cell Adhesion Nanodomains Result from Amyloid Formation on Fungal Cell Surfaces. Peter N. Lipke[1], Melissa C. Garcia[1], Cho Tan[1], Caleen Ramsook[1], David Alsteens[2], and Yves Dufrene[2]. [1]Brooklyn College CUNY and 2Universite Catholique du Louvain

Strong cell-cell adhesive bonds are necessary for fungal homologous interactions in mating and biofilms, and oforheterologous interactions such as pathogen-host binding. Such strong bonds are not characteristic of the low affinity interactions of lectins and low-specificity fungal adhesins, but could form if the adhesin proteins were clustered on the cell surface. Using *Saccharomyces cerevisiae* surface display, we have assayed for activation of cell adhesion in adhesins from *S. cerevisiae* or *Candida albicans.* Atomic Force Microscopy was used to stretch individual *C. albicans* Als5p adhesin molecules on cell surfaces. Stretching caused formation of patches of clustered adhesins on the surface of cell walls. These clusters were visible by confocal microscopy, and were stained with the amyloid dye thioflavin T. Clusters did not form in the presence of amyloid perturbants or an anti-amyloid peptide, or on the surface of cells expressing a non-amyloid mutated form of Als5p. Formation of amyloid surface adhesion domains resulted in activation of cell adhesion and strengthening of adhesive bonds for *C. albicans* Als proteins and for *S. cerevisiae* flocculins, and the activation was also inhibited in the Als5p mutant or in the presence of amyloid perturbants. Thus force-triggered amyloid nanodomain formation activates fungal cell adhesion, and is a property of specific protein sequences in fungal adhesins.
Supported by NIH SCORE Program grant SC1 GM 0853756

DOI: 10.4148/1941-4765.1066

Concurrent Sessions IV

Genome Defense Mechanisms, Epigenetics and RNAi (Aramayo/Liu) **Merrill Hall**

Control of heterochromatin and DNA methylation in *Neurospora crassa*. Eric U. Selker, Shinji Honda, Keyur K. Adhvaryu and Zachary A. Lewis. Institute of Molecular Biology, University of Oregon, Eugene, OR 97403, USA

Most methylated regions of Neurospora are relics of transposons inactivated by RIP (repeat-induced point mutation), a premeiotic homology-based genome defense system that litters duplicated sequences with C:G to T:A mutations. Detailed analyses of the distribution of DNA methylation in the Neurospora genome revealed that it is most concentrated at centromeric regions, subtelomeric regions and disperesed relics of RIP. Our genetic and biochemical studies on the control of DNA methylation revealed clear ties between DNA methylation and chromatin modifications. In vegetative cells, the DIM-2 DNA methyltransferase is directed by heterochromatin protein 1 (HP1), which in turn recognizes trimethyl-lysine 9 on histone H3, placed by the DIM-5 histone H3 methyltransferase. DIM-5 is sensitive to modifications of histones including methylation and phosphorylation and is found in a complex with several other proteins that are essential for DNA methylation: DIM-7, DIM-8 (DDB1), DIM-9 and CUL4. DNA methylation is modulated by a variety of additional factors. For example, mutants in *dmm-1* (DNA methylation modulator-1) show aberrant methylation of DNA and histone H3K9, with both frequently spreading into genes adjacent to inactivated transposable elements. Mutants defective in *dmm-1* grow poorly but growth can be restored by reduction or elimination of DNA methylation using the drug 5-azacytosine or by mutation of the DNA methyltransferase gene, *dim*-2. Mutants defective in both *dmm-1* and *dim-2* display normal H3K9me3 patterns, implying that the spread of H3K9me3 involves DNA methylation. In general, however, HP1 and DIM-2 are dispensable for virtually all H3K9me3. Moreover, H3K9me3 and DNA methylation are rapidly and fully reestablished after these marks are stripped off genetically. I will summarize and discuss our recent progress towards the elucidation of mechanisms controlling heterochromatin and DNA methylation in Neurospora.

Functions of *Mucor circinelloides* RNA-dependent RNA polymerases in the Dicer-dependent and Dicer-independent regulation of endogenous mRNAs. Silvia Calo[1], Juan P. de Haro[1], Francisco E. Nicolás[2], Simon Moxon[3], Santiago Torres-Martínez[1], Tamas Dalmay[2], and Rosa M. Ruiz-Vázquez[1]. [1]Departamento de Genética y Microbiología, Universidad de Murcia, 30100 Murcia, Spain. [2]School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK. [3]School of Computing Sciences, University of East Anglia, Norwich, NR4 7TJ, UK. rmruiz@um.es

The RNA silencing mechanism is involved in the regulation of a number of biological processes through the production of different types of endogenous small RNA molecules (esRNAs), which are usually generated from double-stranded RNA (dsRNA) by Dicer. The esRNA register of fungi is poorly described compared to other eukaryotes and it is not clear what esRNA classes are present in this kingdom and whether they regulate the expression of protein coding genes. Here we report the role of two RNA-dependent RNA polymerase (RdRP) proteins in the esRNAs biogenesis pathways of the basal fungus *Mucor circinelloides*. RdRP-1 and, at a minor extent, RdRP-2 proteins, are required for the production of different classes of esRNAs that are generated with the involvement of a Dicer activity. The largest classes of these esRNAs derive from exons (exonic-siRNAs) and target the mRNAs of protein coding genes from which they were produced. Besides participating in this canonical *dicer*-dependent silencing pathway, *rdrp* genes are involved in a novel degradation process of endogenous mRNAs that is *dicer*-independent. Our results expand the role of RdRPs in gene silencing and reveal the involvement of these proteins in a RNA degradation process that could represent the first step in the evolution of RNA mediated gene silencing.

Genome-wide analysis of *Neurospora crassa* transcripts regulated by the nonsense-mediated mRNA decay pathway. Ying Zhang[1], Fei Yang[1], Mohammed Mohiuddin[2], Stephen K Hutchison[2], Lorri A Guccione[2], Chinnappa Kodira[2], Matthew S Sachs[1]. [1]Texas A&M University, College Station, TX, 77843. [2]Roche 454, Branford, CT, 06405

Nonsense-mediated mRNA decay (NMD) is a surveillance pathway that rids cells of mRNAs that contain premature translation termination codons. It is active in all eukaryotes examined and the core factors are highly conserved. NMD pathways in higher eukaryotes can employ factors that are not present in the yeast *Saccharomyces cerevisiae*, such as components of the exon junction complex (EJC), which has a role in mRNA splicing. The genome of the model filamentous fungus *Neurospora crassa* contains core NMD components as well as EJC components, and, unlike *S. cerevisiae* , many of its mRNAs are spliced. We have established that knockouts of *N. crassa* genes for the NMD components UPF1 and UPF2 lead to the increased stability of specific mRNAs that are NMD substrates. We are using 454 whole transcriptome sequencing to perform studies of transcripts in *N. crassa* strains that are wild-type or deficient in NMD to evaluate at the genome-wide level the changes that occur when this surveillance pathway is eliminated. Here we present the results of our comparative analysis of the whole transcriptome data from wild type and knockout *N. crassa* strains and provide further evidence for the extent and complexity of NMD in regulating transcript metabolism. For example, in the mutant strain, approximately 15% of mRNAs for predicted proteins are at least two-fold up-regulated, and there are a large number of novel exons in the transcriptome.

Domains of DNA methylation in *Coprinopsis cinerea* (*Coprinus cinereus*). Virginia K. Hench and Patricia J. Pukkila. University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA ghench@med.unc.edu

Zemach et al. (Science 328:916-919, 2010) mapped the locations of 5-methylcytosine residues in the *C. cinerea* genome and proposed that repeated sequences, including transposable elements, are targets of DNA methylation in this species. Here we further characterize the domains in this genome in which over 25% of the CpG residues are methylated. These domains (14% of the genome) range in size from 0.3 to 100 kb, and include both repeated (802) and unique (335) genes. One domain on each chromosome includes methylated transposons that have been mapped to the cytological centromere (Stajich et al. PNAS 107:11889-11894, 2010). Since *C. cinerea* has an efficient machinery to detect and methylate tandemly repeated sequences (Freedman et al. Genetics 135:357-366, 1993), we examined the methylation status of several large repeated gene families. Most duplicated paralogs within families such as the Fun K1 kinases, cytochrome P450 genes, and hydrophobins are not methylated. We observe transcription of 54% of all methylated genes, including a DNA methyltransferase gene. Chromosome regions exhibiting elevated rates of meiotic recombination contain a 2.5 fold excess of methylated domains (X^2=116, $P < 0.0001$). These observations raise questions concerning factors that are required to establish and maintain repressive chromatin structures at methylated centromeres, transposon clusters, and genes, but not within other methylated regions. Supported in part by the HHMI through the Precollege and Undergraduate Science Education Program.

Severe symptoms observed for infected RNA silencing mutants of *Cryphonectria parasitica* are associated with a central region of the Hypovirus genome. Xuemin Zhang, Diane Shi and Donald Nuss. Institute for Bioscience and Biotechnology Research, University of Maryland 9600 Gudelsky Dr, Rockville, MD 20850 dnuss@umd.edu

Hypovirus infection of the chestnut blight fungus *Cryphonectria parasitica* attenuates virulence on chestnut trees and induces a wide range of phenotypic changes. Upon virus infection, the RNA silencing pathway of *C. parasitica* is induced as an antiviral defense response. Disruption of the *C. parasitica* RNA silencing antiviral pathway results in very severe symptoms following infection by several mycoviruses, especially the hypovirus type species CHV-1/EP713. In this study, we showed that not all hypoviruses cause severe phenotypic changes of the fungal host in the absence of the RNA silencing pathway. Infection of the *C. parasitica dcl-2* RNA silencing mutant strain with hypovirus CHV- 1/EP721, which is 99% identical to CHV-1/EP713, resulted in symptoms similar to those exhibited by the infected wild-type *C. parasitica* strain. By swapping domains of these two viruses, we mapped the region that is associated with the severe phenotypic change in the *dcl-2* mutant to a 2.5 kb domain located in the central part of the CHV-1/EP713 genome. Chimeric infectious viral cDNA clones carrying this portion of the hypovirus genome caused phenotypic changes in the *dcl-2* mutant similar to that caused by the corresponding parental viruses. The potential for using these chimeric viruses as viral expression vectors is currently being tested.

Diverse Pathways Generate Aberrant RNAs, MicroRNA-like RNAs and Dicer-Independent Small Interfering RNAs in Fungi. Heng-Chi Lee[1], Liande Li[1], Antti Aalto[2], Weifeng Gu[3], Qiuying Yang[1], Craig Mello[3], Dennis Bamford[2], Yi Liu[1]. [1]Department of Physiology, University of Texas Southwestern Medical Center, Texas, USA. [2]Institute of Biotechnology and Department of Biosciences, University of Helsinki, Finland. [3] Program in Molecular Medicine, University of Massachusetts Medical School, USA.

In addition to small interfering RNAs (siRNAs) and microRNAs, several types of endogenously produced small RNAs play important roles in gene regulation. In the filamentous fungus *Neurospora*, the production of qiRNAs requires QDE- 1, QDE-3 and Dicers. Surprisingly, our results suggest that the RNA dependent RNA polymerase (RdRP) QDE-1 is also a DNA-dependent RNA polymerase that produces DNA damage-induced aberrant RNAs (aRNAs), which are the precursor for qiRNA biogenesis. By comprehensively analyzing small RNAs associated with the Argonaute protein QDE-2, we also show that diverse pathways generate miRNA-like small RNAs (milRNAs) and Dicer-independent small interfering RNAs (disiRNAs). milRNAs are processed by at least four different mechanisms that use a combination of Dicers, QDE-2, the exonuclease QIP and a novel RNAse III domain-containing protein MRPL3. disiRNAs originate from loci producing overlapping sense and antisense transcripts, do not require any of the known RNAi pathway components for their production. Taken together, several novel pathways are uncovered, shedding light on the diversity and multi-evolutionary origins of eukaryotic small RNAs.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Mapping and characterization of the Neurospora Spore killer elements. Thomas M. Hammond[1], David G. Rehard[1], Hua Xiao[1], Bryant C. Harris[1], Tony D. Perdue[2], Patricia J. Pukkila[2] and Patrick K. T. Shiu[1]. [1]Division of Biological Sciences, University of Missouri, Columbia, MO, 65211. [2]Department of Biology, University of North Carolina, Chapel Hill, NC 27599

For over 30 years, the Neurospora Spore killers (*Sk*) have been largely known as mysterious DNA elements that span a non-recombining region of 30 cM on chromosome III. In heterozygous crosses between *Sk* and non-*Sk* strains, few non-*Sk* ascospores survive. However, naturally-occurring resistant strains have been found. To elucidate the molecular components of the Neurospora *Sk* system, we took advantage of the ability to rapidly place hygromycin resistance markers at targeted locations with NHEJ (Non-homologous end joining) mutants, for the purpose of mapping the Spore killer resistance gene, *r(Sk-2)*. We are using the knowledge gained by the mapping and characterization of *r(Sk-2)* to identify other key components of the *Sk* system, such as the gene(s) responsible for the spore killing process.

Meiotic SIlencing in Neurospora. Dong Whan Lee, Robert Pratt, Ana Victoria Suescun, Ryan Millimaki, Aldrin Lugena, Alexis Brown, Michelle Yeoman and Rodolfo Aramayo. Department of Biology, Texas A&M University, College Station, TX 77843-3258

In Neurospora meiosis, if a segment of DNA is not present on the opposite homologous chromosome, the resulting "unpaired" DNA segment is targeted for silencing. This situation occurs when a DNA element gets inserted at a particular chromosomal position (e.g., a situation akin to the "invasion" of a genome by transposable DNA elements). It can also occur when a normal region gets deleted. In both situations, the resulting “loop of unpaired DNA” activates a genome-wide "alert" system that results in the silencing not only of the genes present in the "unpaired" DNA segment, but also of those same genes if present elsewhere in the genome, even if they are in the paired condition. This phenomenon is called, meiotic silencing and was originally described in Neurospora crassa, but has since been observed in nematodes and mammals. In all these organisms, "unpaired or unsynapsed" regions (or chromosomes) are targeted for gene silencing. We think that meiotic silencing is a two-step process. First meiotic trans-sensing compares the chromosomes from each parent and identifies significant differences as unpaired DNA. Second, if unpaired DNA is identified, a process called meiotic silencing silences expression of genes within the unpaired region and regions sharing sequence identity. We are using a combination of genetics, molecular biology and biochemistry aimed at identifying all the molecular players of the process and at understanding how they work together. In this work we describe the genetic, molecular, cytogenetic and biochemical characterization of key components of the system: Sms-1 to Sms-17. In addition, we describe components that are essential for the earlier stages of sexual development and discuss the connections between the vegetative pathway Quelling and Meiotic Silencing.

Systems and Synthetic Biology (de Winde/Pentilla) **Fred Farr Forum**

Pathway evolution in Saccharomycetes. Hilary C. Martin, James H. Bullard, Yulia Mostovoy, Sandrine Dudoit, and Rachel B. Brem.

The search to understand how genomes innovate in response to selection dominates the field of evolutionary biology. Powerful molecular evolution approaches have been developed to test individual loci for signatures of selection. In many cases, however, an organism's response to changes in selective pressure may be mediated by multiple genes whose products function together. We have developed methods to detect polygenic evolution across pathways in Saccharomycetes. First, taking classic analyses of allele-specific expression in heterozygotes as a jumping-off point, we have dissected cis- and trans-acting regulatory variants between Saccharomycete species on a genomic scale using Solexa sequencing. With the resulting data sets, we have identified suites of cis-regulatory variants which predominantly upregulate or predominantly downregulate unlinked genes of related function in a given species. Each instance of this directional pattern is a candidate for a model of positive selection, or relaxed purifying selection, on the output of the respective pathway. We have also developed analysis methods for population genomic sequence data in Saccharomycetes, in which we identify pathways with elevated interspecies divergence and reduced polymorphism that reflect a history of selection. Integrating expression- and sequence-based approaches in a study of *S. cerevisiae* and *S. paradoxus*, we have uncovered evidence for a selective sweep of regulatory alleles that tune basal expression and stress responsiveness in a pathway of membrane protein genes.

Genome Regulation in Fission Yeast. Jürg Bähler. University College London, Department of Genetics, Evolution & Environment, London WC1E 6BT, United Kingdom E-mail: j.bahler@ucl.ac.uk; WWW: http://www.bahlerlab.info/

The characteristics of organisms result largely from the dynamic interplay between DNA or RNA and the regulatory apparatus. The control of gene expression is fundamental to implement the information in the genome and to determine the properties of different organisms. Gene expression is regulated at multiple levels, and cells need to integrate external and internal cues and coordinate different regulatory levels to properly exert biological functions. We study transcriptional and post-transcriptional gene expression programmes during cellular proliferation, quiescence/ageing, and stress response using *Schizosaccharomyces pombe* as a model system. We apply multiple genetic and high-throughput approaches for systems-level understanding of regulatory networks and complex relationships between genotype, phenotype, and environment, including roles of genome variation and evolution, transcriptome regulation, and non-coding RNAs.

The development of genetics and genomics for analysis of complex traits in the model filamentous fungus, *Neurospora crassa*. Charles Hall[1], Christopher E. Ellison[1], Elizabeth Hutchison[1], David Kowbel[1], Juliet Welch[1], Rachel B. Brem[2], John W. Taylor[1], N. Louise Glass[1]. Departments of [1]Plant & Microbial Biology and [2]Molecular and Cell Biology, University of California, Berkeley, CA 94720-3102, USA.

Our goal is to develop and make available to the community a set of strains and tools that will facilitate the rapid identification of genes contributing to quantifiable traits in the filamentous ascomycete *Neurospora crassa*, identify regulatory networks on a genomic scale, as well as provide a data-set useful for population genomics. We have used Illumina short-read sequencing of mRNA to simultaneously identify Single Nucleotide Polymorphisms (SNPs) and quantify gene expression for 109 isolates of *N. crassa* from Louisiana USA. The resulting dense marker map has facilitated the mapping of QTLs by association in our wild population with high resolution. Moreover, as most sequence variation in a gene will result in an altered expression level for that gene, combining QTL analyses of physiological and gene expression traits, based on co-localization of expression QTLs (eQTLs) and QTLs can directly indicate candidate genes. We have also utilized this data to identify regulators and their regulatory networks. By this method we will be able to utilize the genetic, phenotypic, and expression variation within a population of *N. crassa* to annotate thousands of previously uncharacterized genes.

A genomics based search for regulators of enzyme production. Tiina Pakula.

Systems-based analysis of adipic acid catabolism in *Penicillium chrysogenum*. Jean-Marc Daran. Delft University of Technology, The Netherlands

Penicillium chrysogenum was successfully engineered to produce a novel carbamoylated cephalosporin that can be used as a synthon for semi-synthetic cephalosporins. To this end, genes for *Acremonium chrysogenum* expandase/hydroxylase and *Streptomyces clavuligerus* carbamoyltransferase were expressed in a penicillinG high-producing strain of *P. chrysogenum*. Growth of the engineered strain in the presence of adipic acid resulted in production of adipoyl-7-amino-3-carbamoyloxymethyl-3-cephem-4-carboxylic acid (ad7-ACCCA) and of several adipoylated pathway intermediates. However, only a small fraction (circa 4%) of the consumed adipic acid was recovered as ad7-ACCCA. A combinatorial chemostat-based transcriptome study, in which the ad7-ACCCA-producing strain and a strain lacking key genes in beta-lactam synthesis were grown in the presence and absence of adipic acid, enabled the dissection of transcriptional responses to adipic acid per se and to ad7-ACCCA production. Transcriptome analysis revealed that adipate catabolism in *P. chrysogenum* occurs via beta-oxidation and enabled the identification of putative genes for enzymes involved in mitochondrial and peroxisomal beta-oxidation pathways. Analysis of expressed genes encoding putative acyl-CoA oxidases and dehydrogenases provide more detailed information on the molecular mechanisms involved in adipic acid catabolism.

Hyphal Heterogeneity in *Aspergillus niger*. Charissa de Bekker, Arman Vinck, Han Wösten Microbiology and Kluyver Centre for Genomics of Industrial Fermentations, Utrecht University, The Netherlands

Mycelia of filamentous fungi explore new substrates by means of growing hyphae. These hyphae secrete enzymes that degrade organic material into small molecules that can be taken up to serve as nutrients. Previously, it has been shown that only part of the exploring hyphae of *Aspergillus niger* highly express the glucoamylase gene *glaA*. This was a surprising finding considering the fact that all hyphae were exposed to the same environmental conditions. Using reporter studies, we have demonstrated that the expression of other secretion enzyme encoding genes in *A. niger* is also heterogenic. Co-expression studies showed that hyphae that highly express one of these genes also highly express other genes encoding secreted proteins. Over and above this, high expression of genes encoding secreted proteins correlated with high expression of a gene involved in central metabolism and with high ribosomal RNA content. This suggests that there are populations of hyphae at the periphery that differ in their transcriptional and translational activities. These studies were extended with whole genome transcription profiling of individual hyphae. In order to perform (sub)-cellular transcriptomics on single exploring hyphae, protocols have been set up to collect individual hyphae using LPC, isolate RNA and amplify cDNA. Microarray analysis led to the conclusion that exploring neighboring hyphae are highly heterogenic in gene expression. Genes with heterogenic expression can be found in all functional gene classes.

Genome-scale metabolic reconstruction and curation of the filamentous fungi *Neurospora crassa*. Jeremy Zucker1, Heather Hood2, Jonathan Dreyfus3, and James Galagan3. 1Broad Institute of MIT and Harvard, Cambridge MA 2OHSU, Portland, OR 3 Boston University, Boston, MA

We present NeurosporaCyc, a genome-scale flux balanced model of the filamentous fungi *Neurospora crassa* that is capable of representing, integrating, modeling and simulating the data avalanche of omics data being generated for this organism. We constructed this model using a combination of automated inference and manual curation based on the extensive literature for Neurospora. Furthermore, we have experimentally validated the growth phenotypes predicted by this model.

Systems-level design of filamentous fungi- integration of *in silico* flux modes and in-vivo pathway fluxes towards desired production properties. Guido Melzer, Habib Driouch and Christoph Wittmann. Institute of Biochemical Engineering, Technische Universität Braunschweig, Germany

The recent efforts in genome sequencing and genome-scale modelling are major drivers for the prediction of genetic targets and optimal pathways towards superior cell factories. On basis of this rich set of information, computational and experimental strategies now aim at systems-wide optimization of tailor-made filamentous fungi (Melzer et al., 2009). Here, we report on a novel approach recruiting computationally and experimentally derived flux information for metabolic design of superior production properties. On basis of a large-scale metabolic model of *Aspergillus niger*, functionally condensed from genome-scale, elementary flux modes were determined for different scenarios of interest. Hereby, a novel approach screened the large set of elementary modes, each representing a unique flux distribution for flux correlations between metabolic reactions (Melzer et al., 2009). This, identified reactions coupled to desired properties such as production fluxes and thus, for the first time allowed the simultaneous prediction of deletion and amplification targets. Exemplified for different industrially relevant cell factories, products such as recombinant enzymes or chemicals and raw materials such as sugars, hemicelluloses or oils, the simulations revealed that the success of identification of most of the genetic targets depends on the differentiation of biological states, i.e. growth-associated or non-growth-associated formation of the target product. In addition, selected targets, such as the pathways of protein synthesis seem independent of the biological state. The simulation results nicely match with experimental data, comprising in vitro enzyme assay and experimental flux data. The integrated strategy of combining computational and experimental systems biology seems of high relevance for future metabolic engineering of fungal cell factories.

Melzer G, Esfandabadi ME, Franco-Lara E, Wittmann C: Flux Design: *In silico* design of cell factories based on correlation of pathway fluxes to desired properties. BMC Syst Biol 2009, 3:120.

Fungi that Infect Humans (Mitchell/Lodge) **Chapel**

Hsp90 Governs Drug Resistance and Dispersion of Fungal Biofilms Nicole Robbins[1], Priya Uppuluri[2], Jeniel Nett[3], Ranjith Rajendran[4], Gordon Ramage[4], Jose L. Lopez-Ribot[2], David Andes[3], and Leah E. Cowen[1*] [1]Department of Molecular Genetics, University of Toronto, Toronto, ON M5S 1A8, Canada; [2]Department of Biology and South Texas Center for Emerging Infectious Diseases, University of Texas at San Antonio, Texas, 78249, USA; [3]Department of Medicine, University of Wisconsin, Madison, Wisconsin, 53792, USA; [4] Glasgow Dental School and Hospital, University of Glasgow, Glasgow, G2 3JZ, UK

Fungal biofilms are a major cause of human mortality and are recalcitrant to most treatments due to intrinsic dug resistance. These complex communities of multiple cell types form on indwelling medical devices and their eradication often requires surgical removal of infected devices. Here we implicate the molecular chaperone Hsp90 as a key regulator of biofilm drug resistance and dispersion, with profound therapeutic potential. We previously established that in the leading human fungal pathogen, *Candida albicans*, Hsp90 enables the emergence and maintenance of drug resistance in planktonic conditions by stabilizing the protein phosphatase calcineurin and MAPK Mkc1. Hsp90 also regulates temperature-dependent *C. albicans* morphogenesis through repression of cAMP-PKA signaling. Here we demonstrate that genetic or pharmacological compromise of Hsp90 had negligible impact on *C. albicans* biofilm growth and maturation in vitro but abrogated resistance to the most widely deployed antifungal drugs, the azoles. Although biofilms were resistant to the Hsp90 inhibitor geldanamycin or the azole fluconazole individually, there was potent synergy with the drug combination. Genetic depletion of Hsp90 rendered biofilms exquisitely sensitive to fluconazole, with >100-fold increase in sensitivity in vitro. Depletion of Hsp90 transformed fluconazole from ineffectual to highly effective in eradicating biofilms in a rat venous catheter model of infection. Depletion of Hsp90 also reduced dispersal of biofilm cells, blocking their capacity to serve as reservoirs for infection. Reduction of Hsp90 levels led to depletion of calcineurin and Mkc1 in planktonic but not biofilm conditions, suggesting that Hsp90 regulates drug resistance through different mechanisms in these distinct cellular states. Inhibition of Hsp90 also reduced resistance of biofilms of the most lethal mould *Aspergillus fumigatus* to the only new class of antifungal drugs to reach the clinic in decades, the echinocandins. Thus, targeting Hsp90 provides a much-needed strategy for improving clinical outcome in the treatment of biofilm infections. N.R. is supported by an NSERC CGS-D and L.E.C. by a BWF CABS, CRC, and CIHR MOP-86452.

A systems approach to regulation of a fungal virulence factor. Haynes, Brian C.[1], Skowyra, Michael L.[2], Gish, Stacey R.[2], Williams, Matthew[2], Brent, Michael R.[1],and Tamara L. Doering[2]. [1]Department of Computer Science, Washington University School of Engineering, St. Louis, MO, USA. [2] Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA.

Cryptococcus neoformans is a pathogenic yeast responsible for serious opportunistic infections that lead to over 600,000 deaths annually worldwide. The major virulence factor of this fungus is a polysaccharide capsule, which is dramatically regulated by growth conditions. Elegant work by several groups working in this field has identified a handful of transcription factors that impact capsule phenotype. However, a more global picture of the relationships between these factors is lacking. We have combined transcriptional and phenotypic profiling to model the capsule regulatory network in *C. neoformans*. This approach has implicated an array of putative transcription factors in capsule regulation, and uncovered relationships between several of these and known regulators. Deletion of the gene encoding one putative transcription factor, Bch1p, yields cells with a notable reduction in capsule size and loss of virulence in animal models. Progress on this project and this rapidly developing area will be presented. This work is supported by NIH grant GM071007.

Allergen 1 and 2 constitute a novel class of virulence associated genes that are regulated by phenotypic switching in *Cryptococcus neoformans*. Neena Jain, Radames J. Cordero, Tejas Gawade and Bettina C. Fries. Department of Medicine, Microbiology and Immunology Albert-Einstein College of Medicine Bronx NY

Cryptococcus neoformans, a human pathogenic fungus is a major cause of mortality and morbidity in immunocompromised patients. Cryptococcal infections are characterized by a complex relationship between the host and the pathogen referred to as patho-adaptation. *C. neoformans* can undergo phenotypic switching from a smooth (SM) to a mucoid (MC) colony switch variant during chronic infection. Prior work has associated this switch with virulence including high intracranial pressure and the ability to persist in the host. We have determined that the switch of the SM parent to the hypervirulent MC variant is associated with down-regulation of several genes, among them *Allergen1* and *Allergen 2* (*ALL1* and *ALL2)*. These genes encode for highly homologous cytosolic proteins of unknown function. Deletion mutants of these proteins, namely *all1D* and *all2D*, mimic the hypervirulent phenotype of the MC variant. Especially the *all1D* mutant has a striking phenotype, as it is more virulent than the SM parent in pulmonary infection and results in augmented intracranial pressure in a CNS infection model. Most interestingly, static and dynamic light scatter analysis demonstrates that the *all1D* sheds a capsular polysaccharide with altered shape that results in changes of viscosity. This mutant also exhibits impaired capsule induction. Since capsular polysaccharide is implicated in high intracranial pressure we now have a mechanistic link between regulation of *ALL1* and the changes in virulence. In addition to changes in the capsular polysaccharide the loss of *ALL1* and *ALL2* makes the mutants more resistant to stress from oxygen radical and affects their replicative life span. In summary phenotypic switching in *C. neoformans* augments its virulence by down regulation of two genes that play a key role in polysaccharide biology, stress resistance and lifespan. These findings highlight that virulence in this pathogenic yeast is a dynamic property that can be altered by down regulation of *ALL1* and *ALL2*.

Chitosan is necessary to establish *Cryptococcus neoformans* infection. Lorina G. Baker Boomhower[1], Charles A. Specht[2], and Jennifer K. Lodge[1] Department of Molecular Microbiology[1], Washington University School of Medicine, 660 S. Euclid Avenue, Saint Louis, Missouri 63110, and Department of Medicine[2], University of Massachusetts, 364 Plantation Street, Worcester, Massachusetts 01605

Cryptococcus neoformans is an opportunistic pathogen that mostly infects immunocompromised individuals. Its cell wall is an essential organelle that provides structure and integrity. Several known virulence factors are located or attached to it, including melanin, phospholipase, and the polysaccharide capsule. The wall matrix is a complex structure composed of chitin, chitosan, and glucans. Chitin is an indispensable component with the majority converted to the deacetylated form, chitosan, by three chitin deacetylases (Cda1, Cda2, and Cda3). The deletion of all three-chitin deacetylease results in loss of chitosan production. In a mouse model the triple chitin deacetylse deletion strain was avirulent and did not establish infection. Additionally, both the chitin synthase three and chitin synthase regulator two deletion strains, each with negligible chitosan levels, had similar in vivo phenotypes. Together the data indicated chitosan is necessary for in vivo growth. Interestingly, the single deletion of *CDA1* resulted in attenuated virulence and reduced fungal burden, which suggested it or the chitosan produced by it is needed for virulence. Collectively the data suggest the proteins involved in chitosan synthesis may be good targets for anti-cryptococcal therapeutics.

Telomeres and Cell Wall Proteins in *Candida glabrata.* Brian Green, Nicole Benoit, Elizabeth Hwang, Margaret Zupancic, Brendan Cormack. Department of Molecular Biology and Genetics. Johns Hopkins University School of Medicine

Candida glabrata is an important agent of both mucosal and disseminated candidiasis. At the genome levels, *Candida* shows a high degree of similarity to the non-pathogenic *Saccharomyces cerevisiae*. Comparative genomics reveals approximately 300 genes present in *C. glabrata* that are absent in *S. cerevisiae*; many of these are encoded in sub-telomeric regions of the genome and the majority of these encode cell surface proteins. *C. glabrata* is able to adhere avidly to human cells. Adherence is dependent primarily on a family of cell wall proteins, encoded by the *EPA* genes. Multiple members of this family can mediate adherence to glycans expressed on epithelial or endothelial cells. The Epa proteins are GPI anchored cell wall proteins (GPI-CWPs), crosslinked to cell wall glucan via a remnant of the GPI anchor. This class of protein constitutes the major class of cell wall localized protein and it is likely that the vast majority of proteins expressed at the surface of *C. glabrata* are also GPI-CWPs.

In order to characterize the full complement of *C. glabrata* cell surface proteins, we have undertaken a careful analysis of the sub-telomeric regions of *C. glabrata*. Sub-telomeres are rich in repeated regions and assembly of the sub-telomeres from shotgun sequences is challenging; it is recognized that the current genome assembly of these regions is imperfect. We have taken the approach of individually cloning the sub-telomeric regions for all 13 chromosomes and sequencing these clones. We have carried out this analysis for two strains, the sequenced type strain ATCC2001 (CBS138) and BG2, our lab strain. For these two strains, divergence is relatively high, approximately 0.5% nucleotide divergence across the genome as a whole. We have successfully cloned, sequenced and assembled most of the sub-telomeres from both strains and will report on insights regarding the structure of the sub-telomeres and the complement of GPI-CWPs in *C. glabrata*.

Strikingly, most sub-telomeres end in a characteristic structure with a GPI-CWP gene followed by 5-7 kb of non coding sequence. These terminal genes, annotated previously as pseudogenes, are almost invariably transcribed towards the telomere. A second insight has come from comparison of sub-telomere structure between the two sequenced strains. For most of the sub-telomeric region, the sub-telomeres are largely syntenic between the two strains with only a few examples of large scale translocations, gene insertions or deletions between the strains. In contrast to the bulk of the sub-telomeric region, the last 5-7 kb of the sub-telomeres shows substantial divergence between the two sequenced strains, suggesting a potential for rapid microevolution in these regions of the genome.

Analysis of the secretomes of *Cryptococcus gattii* strains with different virulence profiles. Leona T. Campbell[1], Elizabeth Harry[2], Ben Herbert[3] and Dee A. Carter[1]. [1]School of Molecular Bioscience, University of Sydney, Sydney, NSW, Australia [2] iThree Institute, University of Technology, Sydney, NSW, Australia [3]Department of Chemistry & Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia

Cryptococcus gattii is capable of causing disease in a wide range of animal hosts. Closely related strains of *C. gattii* exhibit significant differences in virulence in mammalian hosts. As fungi produce a range of secreted degrative enzymes, and as these may invoke a host response, the fungal secretome is likely to be important in modulating host- pathogen interaction. In this study, we compare the secretomes of two *C. gattii* strains, one categorized as hypervirulent (R265) and the other exhibiting low-level virulence (R272). A total of 27 proteins were identified with only four proteins being shared between strains. The secretome of R265 primarily included uncharacterized proteins containing catalytic cores with roles in carbohydrate degradation as well as the antioxidant superoxide dismutase and a GTPase. R272 secreted a more diverse set of proteins including enolase and transaldolase, enzymes canonically involved in glycolysis and the pentose phosphate pathway respectively, but both also described as fungal allergens that bind IgE. This work indicates that different classes of proteins are secreted by closely related strains of *C. gattii* exhibiting different levels of virulence.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Iron is critical for mucormycosis pathogenesis in the diabetic ketoacidotic host. Ashraf S. Ibrahim, Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, Torrance, CA

Mucormycosis is a life-threatening infection that occurs in patients who are immunocompromised due to diabetic ketoacidosis (DKA), neutropenia, organ transplantation, and/or increased available serum iron. Despite aggressive therapy the overall mortality is high and the incidents of mucormycosis are on the rise.
Clinical and animal model data demonstrate that the presence of elevated available serum iron (e.g. as seen in DKA patients) predisposes the host to develop mucormycosis which is commonly caused by *Rhizopus oryzae*. Therefore, abrogation of fungal iron acquisition is a promising therapeutic strategy to impact clinical outcomes for this deadly disease. The high affinity iron permease gene (*FTR1*) is required for *R. oryzae* iron transport in iron-limited environments in vitro and for full virulence in mice as determined by gene disruption and RNA-interference studies. Passive immunization with anti-Ftr1p immune sera protects DKA mice from infection with *R. oryzae*.
Another hallmark of mucormycosis, is the angioinvasion nature of infection. We identified GRP78 as a novel host receptor which mediates invasion and damage of endothelial cells by Mucorales. Elevated concentrations of glucose and iron, consistent with those seen during DKA, enhance GRP78 expression and resulting invasion and damage of endothelial cells in a receptor-dependent manner. Anti-GRP78 immune serum protects mice in DKA from mucormycosis. Collectively, these results show that iron plays a critical role in mediating *R. oryzae* virulence in the DKA host.

Insight into transcriptional regulatory mechanisms controlling filamentation in *Candida albicans* under hypoxia. Adnane Sellam, André Nantel, Faiza Tebbji, Christopher Askew, Malcolm Whiteway. McGill University, Montreal, PQ, Canada.

To gain insight into regulatory mechanisms controlling hyphae formation in response to low oxygen concentration in the opportunistic yeast *C. albicans* a compilation of mutants from various publicly available libraries were screened (648 mutant strains). In this work we focused our investigation on mutants of genes encoding for transcription factors, components of chromatin remodeling and histone modification complexes, and protein acting in different signaling pathways. The ability of mutants to form hyphae specifically under hypoxic condition was assessed in solid medium by scoring the filamentation of colonies peripheral regions. Filamentation screen identified 40 mutants with substantial morphology defect. We focused our investigation on the transcription factor Ahp1p whose mutant displayed a hyperfilamentation specifically under hypoxia. Transcriptional regulatory network associated with this factor was characterized using both expression profiling and ChIP-chip. The obtained results demonstrated that this factor is required to activate genes involved in iron uptake and many adhesin-encoding genes specifically under hypoxia. Transcriptional regulatory circuits implicating other regulators will be also presented and their role in the adaptation to hypoxia and filamentation will be discussed.

Proteomics and Metabolomics (Solomon/Hammond-Kosack) **Kiln**

A metabolomic study of *Candida albicans* morphogenesis reveals the potential role of the cell redox balance on the morphological transition. **Silas G. Villas-Bôas[1]**, Ting-Li Han[1] & Richard D. Cannon. [1]Centre for Microbial Innovation, School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand; [2]Department of Oral Sciences, The University of Otago, PO Box 647, Dunedin, New Zealand.

Candida albicans is a polymorphic fungus that has the remarkable ability to rapidly switch between yeast and hyphal forms in response to various environmental factors (e.g. quorum sensing molecules). This morphological transition is considered to be a critical virulence factor of this fungus. The metabolic mechanisms that recognize environmental signals and promote the morphological changes at a system level, however, remain unclear. In this study, we investigated the metabolic changes during the morphogenesis of *C. albicans* under laboratory conditions using metabolomics and isotope-labelling experiments. Our results demonstrated that 16 metabolic pathways involved in the central carbon metabolism were significantly up-regulated when *C. albicans* changed from yeast to hyphal form, whilst 11 metabolic pathways were downregulated when hyphal formation was suppressed by specific quorum sensing molecules (farnesol and phenylethyl alcohol). Combining the results from isotope-labelling experiment with metabolomics data, a final shortlist of 2 metabolic pathways was obtained. These pathways highlighted the important role of $NADP^+$/NADPH in the global regulation of *C. albicans* morphogenesis. The importance of each pathway on the morphological switch of *C. albicans* is being validated by gene knockout mutagenesis.

Metabolome phenotyping of *Fusarium graminearum* wt and single gene deletion mutant strains affected in virulence under DON-inducing and non-inducing conditions. Rohan Lowe[1], Martin Urban [1], Gail Canning[1], William Allwood[1], Mike Beale[2], Jane Ward[2] and Kim Hammond-Kosack[1] [1]Centre for Sustainable Pest and Disease Management. [2] National Centre for Plant and Microbial Metabolomics; Rothamsted Research, Harpenden, Herts., AL5 2JQ, United Kingdom. Email: Martin.Urban@bbsrc.ac.uk

Fusarium graminearum causes plant disease on cereal crops including wheat, barley and maize. The fungus reduces yield and contaminates the crop with secondary metabolites toxic to plants and animals. These metabolites include trichothecene mycotoxins such as deoxynivalenol (DON). We recently reported on the use of metabolomic analysis to understand the basal metabolism in four Fusarium spp., *F. graminearum*, *F. culmorum*, *F. pseudograminearum* and *F. venenatum* under DON and non-DON inducing conditions (Lowe et al., MPMI, 2010, 16005-1618). In this study we investigated the global metabolic changes during time course experiments in the *F. graminearum* wt strain PH-1 for which the complete genomic sequence is available (http://www.broad.edu). Also, a 'triple-fingerprint' of analytical techniques including 1H-NMR and electrospray mass-spectroscopy (+/- ESI-MS) was recorded to characterise several single gene deletion mutants affected in mycotoxin biosynthesis, cell signalling and plant pathogenicity. Interestingly, all mutants analysed so far showed significant changes in primary metabolism. Understanding these changes will require more in-depth understanding of the metabolic networks and the characterisation of the metabolites present in *F. graminearum*. Rothamsted Research receives grant aided support from the Biotechnology and Biological Sciences Research Council.

The fludioxonil induced phosphoproteomes of the phytopathogenic fungi *Alternaria brassicicola* and *Botrytis cinerea*. Marlène Davanture[1], Benoît Valot[1], Claire Campion[2], Jérôme Dumur[2], Nelly Bataillé-Simoneau[2], Michel Zivy[1], Philippe Simoneau[2], and S. Fillinger[3] [1]PAPPSO, INRA-CNRS-University Paris XI-Agro ParisTech, Gif-sur-Yvette, France; [2]UMR PaVé, INRA-University Angers, France; [3]BIOGER CPP, INRA Versailles-Grignon, France

Protein phosphorylation and dephosphorylation are cellular processes rapidly induced by external *stimuli* adapting regulatory circuits and enzymatic functions to changing environmental conditions. Signal transduction (ST) pathways necessary for signal perception and phosphorylation cascades are involved in many physiological processes such as development, stress adaptation, virulence etc. ST components may either constitute targets for agronomic fungicides or mediate resistance to these compounds. In order to identify and to compare the proteins involved in transducing the signal perceived after phenylpyrrol treatment in two phytopathogenic fungi, we established a gel-free phosphoproteomic approach for systematic identification of phosphorylated peptides in *Alternaria brassicicola* and *Botrytis cinerea*, treated or not with fludioxonil. The gel-free phosphoproteomic approach combines two sequential steps after trypsin digestion : i) SCX chromatography (strong cation exchange); ii) IMAC (Immobilized metal affinity chromatography) for the enrichment of phosphopeptides prior to LC-MS/MS analysis. Preliminary experiments revealed that among the high number of identified phosphoproteins (500-600 and >800 for *Ab* and *Bc* samples, respectively), more than 12% were found specifically phosphorylated and 8% dephosphorylated following the fludioxonil treatment. Among the functional categories identified, we noticed a high proportion of proteins with regulatory functions (transcription, translation etc.) or involved in signal transduction. Particular cellular functions affected by (de)phosphorylation under these conditions concern the cell envelope and transport across it. Higher proportions of phosphorylated proteins compared to dephosphorylated proteins following fludioxonil treatment were found for the functional categories of metabolism and energy production, especially lipid metabolism, as well as of cytoskeleton and cell cycle. We are currently analyzing additional samples to confirm this analysis.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

A functional genomics study of extracellular protease production by *Aspergillus niger*. Machtelt Braaksma[1], Mariet van der Werf[1], Cees van den Hondel[1,2] and Peter Punt[1,2] 1, TNO Microbiology and Systems Biology, Zeist , the Netherlands 2, Molecular Biology and Biotechnology, Leiden University

To study the complex regulation of extracellular proteases in the filamentous fungus *Aspergillus niger* a combination of comprehensive functional genomics technologies was used. The requirements for performing a successful systems biology study and addressing the challenges met in analyzing the large, information-rich data sets is adressed. Protease activity plays an important role in strain and process development of *A. niger* and other aspergilli. In our research the influence of several environmental factors on the production of extracellular proteases in controlled batch cultivations was studied. Samples generated in this study were used for analysis with functional genomics technologies. With a shotgun proteomics approach the *A. niger* secretome under different experimental conditions was determined. Furthermore, the effect of different quantitative phenotypes related to protease or glucoamylase activity on the information content of a metabolomics data set was investigated. Based on a transcriptomics study the identification of co-expression networks is described. First, a set of conserved genes was used to construct these networks. Subsequently, all annotated genes, including hypothetical and poorly conserved genes, were integrated into the co- expression analysis, allowing identification of novel targets for strain and process development.

A proteomics approach to understanding virulence in *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat. S.E. Strelkov, Y.M. Kim, T. Cao, and N.N.V. Kav. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada. E-mail: stephen.strelkov@ualberta.ca

The fungus *Pyrenophora tritici-repentis* causes tan spot, an important foliar disease of wheat. This pathogen produces three host-specific toxins, including Ptr ToxB, a small chlorosis-inducing protein. It has been suggested that the virulence conferred by the Ptr toxins is superimposed on the general pathogenic ability of *P. tritici-repentis*. A proteomics approach was employed to better understand the mode of Ptr ToxB action in toxin-sensitive wheat, and to evaluate whether or not there are additional differences, beyond toxin production, between virulent and avirulent isolates. Analysis of the wheat leaf proteome by 2-DE revealed 102 protein spots with significantly altered intensities, relative to buffer-treated controls, after toxin-treatment but prior to the development of visible chlorosis. The identities of 47 of these spots were established by MS/MS and included proteins involved in the light reactions of photosynthesis, the Calvin cycle, and the stress/defense response. These changes were accompanied by a rapid decline in photosynthesis, as measured by infrared gas analysis. Thus, it seems that Ptr ToxB disrupts the photosynthetic process in sensitive wheat, leading to chlorophyll photooxidation and chlorosis. Comparisons of the secretome and mycelial proteome of the virulent and avirulent isolates revealed 133 differentially abundant proteins, 63 of which were identified by MS/MS. A number of the up-regulated proteins in the virulent isolate have been implicated in microbial virulence in other pathosystems, suggesting an enhanced general pathogenic ability in this isolate irrespective of toxin production. Collectively, these results highlight the utility of proteomic studies in increasing understanding of the tan spot pathosystem.

The *Cryptococcus gattii* proteome in growth and response to fluconazole. Hin Siong Chong[1], Leona Campbell[1], Ben Herbert[2], Elizabeth Harry[3], Mark Krockenberger[4], Marc Wilkins[5] and Dee Carter[1]. [1] School of Molecular Bioscience, Sydney University, Australia; [2] Chemistry and Biomolecular Sciences, Macquarie University, Sydney, Australia; [3] iThree Institute, UTS, Sydney, Australia; [4] Department of Veterinary Pathology, Sydney University, Australia; [5] School of Biotechnology and Biomolecular Sciences, UNWS, Sydney, Australia. dee.carter@sydney.edu.au

Cryptococcus gattii is a pathogenic yeast capable of causing disease in immunocompetent people. Antifungal susceptibility testing has found closely related strains of *C. gattii* can vary greatly in their susceptibility to fluconazole (FLC) and other azoles, without any apparent prior exposure to these drugs. Our group is interested in determining the molecular basis of the antifungal response in *C. gattii*. To establish a baseline for this work we have examined the proteome of *C. gattii* cells that are moderately susceptible to FLC during normal growth and in the presence of FLC. Compared to normal growth, cells treated with FLC had reduced levels of ribosomal proteins and increased stress-related proteins, including several heat shock proteins. A number of proteins involved in ATP biosynthesis also increased, indicating that ATP-dependent efflux of FLC and other toxic metabolic byproducts had been initiated. Ongoing studies will determine how the proteome compares in strains with elevated FLC resistance.

A quantitative proteomic analysis of the wheat pathogen *Stagonospora nodorum* during sporulation. Liam Cassidy[1, 2], Richard Oliver, Richard Lipscombe[2], Peter Solomon[1]. [1]Research School of Biology, The Australian National University, Canberra 0200, ACT, Australia. [2]Proteomics International Pty Ltd, PO Box 3008, Broadway, Nedlands 6009, WA, Australia

Stagonospora nodorum is an important fungal pathogen of wheat and the causal agent of the disease Stagonospora nodorum blotch. During the wheat growing season *S.nodorum* undergoes multiple rounds of asexual sporulation. This polycyclic lifecycle enables it to propagate on and across crops resulting in accumulation of the pathogen on the plants, and accentuation of the damage it inflicts.
Previously, studies using various molecular techniques have looked in-depth into the genomics and metabolomics of *S.nodorum* during the infection of wheat with the aim of finding specific targets that will aid in the control of this pathogen. These studies have identified a number of independent pathways with possible key roles in the process of sporulation.
This study utilises shotgun proteomic techniques to characterise the proteome of *Stagonospora nodorum* and three mutant strains (with disruptions in pathways previously shown to compromise sporulation) at time points prior to, and during, sporulation.
In order to analyse the proteomes of the four strains at two time points quantitatively, we have neutilised iTRAQ 8-plex isobaric peptide tagging technology coupled with 2D LC-MS/MS.
Following analysis of the proteomics data, we are now utilising molecular biology techniques to knock out genes encoding proteins found to be differentially abundant during the process of sporulation. By creating knock-out mutants lacking these proteins and characterising the growth we will be able to further elucidate and confirm pathways that play key roles in sporulation for this important wheat pathogen.

A Mass Spectrometry Based Examination of the Magnaporthe oryzae Proteome During Appressorium Development. William L Franck[1], Emine Gokce[2], Yeonyee Oh[1], Timothy S. Collier[2], David C. Muddiman[2] and Ralph A. Dean[1] [1]Center for Integrated Fungal Research, [2]W.M. Keck FT-ICR Mass Spectrometry Laboratory, Department of Chemistry, North Carolina State University, Raleigh, North Carolina, 27606

The rice blast pathogen, *Magnaporthe oryzae*, penetrates leaf surfaces via infection structures known as appressoria. Appressorium formation can be induced by germination of conidia on hydrophobic surfaces or hydrophilic surfaces in the presence of cAMP. To better understand the physiological changes occurring during the formation of appressoria, a mass spectrometry-based proteomics study was initiated to examine changes in the proteome during spore germination and appressorium development. 1393 proteins were identified from five conditions including, conidia and conidia germinated on a hydrophilic surface for 4 and 18 hours in the presence or absence of 50mM cAMP. A detailed examination of this data including changes in protein abundance will be presented. In addition, an analysis of the phosphoproteome is in progress. Protein kinase dependent signaling is indispensible for production of appressoria. Phospho-(Ser/Thr) kinase substrate antibodies were used to observe kinase-specific changes in protein phosphorylation patterns between mycelium, conidia and germinated conidia forming appressoria. These results provide a foundation for examining the phosphoproteome and identifying protein kinase targets involved in appressorium development.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Signaling mechanisms that sense and combat oxidative stress in *Schizosaccharomyces pombe*. Kaz Shiozaki, Department of Microbiology, University of California, Davis, CA 95616, USA and Graduate School of Biological Science, Nara Institute of Science and Technology, Ikoma, Nara 630-0192, JAPAN

Oxidative stress that generates the reactive oxygen species (ROS) is one of the major causes of DNA damage and mutations. The "DNA damage checkpoints" that arrest cell cycle and repair damaged DNA have been a focus of recent studies. However, means to eliminate ROS are likely to be as important as the DNA repair mechanisms in order to suppress mutations in the chromosomal DNA. To understand how eukaryotes combat oxidative stress, our laboratory utilizes the fission yeast *Schizosaccharomyces pombe*, which has consistently served as an excellent model system to uncover the cellular mechanisms broadly conserved among eukaryotes, including humans. Mechanisms that sense and transmit oxidative stress signals to the stress MAP kinase cascade and the TOR (Target Of Rapamycin) signaling pathway in fission yeast will be discussed.

The CCAAT-binding complex coordinates the oxidative stress response in eukaryotes.
Axel A. Brakhage[1], Marcel Thön[1], Qusai A. Abdallah[1], Daniel H. Scharf[1], Martin Eisendle[2], Hubertus Haas[2], and Peter Hortschansky[1]
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The heterotrimeric CCAAT-binding complex is a global regulator in all eukaryotes, but the regulation of its activity is still poorly understood. The *Aspergillus nidulans* CCAAT-binding factor (AnCF) consists of the subunits HapB, HapC and HapE and senses the redox status of the cell via oxidative modification of thiol groups within the histone fold motif of HapC. Mutational and *in vitro* interaction analyses revealed that two of these cysteine residues are indispensable for stable HapC/HapE subcomplex formation, high affinity DNA-binding and proper nuclear import of AnCF. Oxidized HapC is unable to participate in AnCF assembly and localizes in the cytoplasm, but can be recycled by the thioredoxin system *in vitro* and *in vivo*. In this study, we demonstrate that the central transcription factor AnCF is regulated at the post-transcriptional level by interconnected feedback loops with the peroxide sensor NapA. AnCF represses full expression of *napA* and some NapA target genes. Oxidative stress inactivates AnCF via oxidation of HapC, which increases expression of *napA* and NapA target genes directly (via release of AnCF repression) and indirectly (via NapA activation). This response includes the activation of the thioredoxin system, which represses NapA activity and reactivates AnCF. The coordinated activation and deactivation of antioxidative defense mechanisms, i.e., production of enzymes such as catalase, thioredoxin or peroxiredoxin, and maintenance of a distinct glutathione homeostasis very likely represents a evolutionary conserved regulatory feature of the CCAAT-binding complex in eukaryotes.

On the role of NOX-derived ROS during cell fusion in *Neurospora crassa*. Alexander Lichius and Nick Read, Edinburgh University, UK, alexlichius@gmail.com

Deletion of NADPH-oxidase-1 (NOX-1), its regulator NOR-1, and the associated GTPase RAC-1, resulted in a complete loss of cell fusion in *Neurospora crassa*, whereas deletion of NOX-2 did not, confirming functional separation between both isoforms. Although *nox-1* and *nor-1* cells retained the ability to form conidial anastomosis tubes (CATs) they were unable to chemotropically interact. Ectopic expression of fluorescent NOX-1 and NOR-1 fusion constructs rescued both mutant phenotypes, and their localisation to internal membranes and the cytoplasm, respectively, indicated a role for NOX-1-derived reactive oxygen species (ROS) in intracellular redox signalling. This notion was supported by the fact that CAT formation was selectively inhibited through the addition of micromolar concentrations of hydrogenperoxide, which left germ tube development unaffected. Visualization of superoxide accumulation in the tips of *nox-1* and *nor-1* germ tubes suggested that NOX-1 activity is dispensable for polarized growth, but has specific functions during CAT-mediated cell fusion. Deletion of the catabolic NAD-dependent glutamate dehydrogenase (GDH-1(NAD)) resulted in a phenotype very similar to *nox-1*, whereas absence of its anabolic counterpart NADPH-dependent GDH (GDH(NADPH)) produced no obvious phenotype. Taken together, this data suggests that activity of GDH-1(NAD) is required to replenish NADPH stores in order to fuel NOX-1-mediated ROS production which is essential to induce morphogenetic transitions leading to cell fusion.

The *HYR1* gene in the rice blast fungus functions to tolerate plant-produced reactive oxygen species during infection. Kun Huang[1], James Sweigard[2], Jeffrey Caplan[3], Kirk Czymmek[1], Nicole Donofrio[1]. [1]University of Delaware, Newark, DE, 19716 [2]DuPont Stine-Haskell, Elkton Rd, Newark, DE, 19711 [3]Delaware Biotechnology Institute, Newark, DE, 19716 ndonof@udel.edu

Plants can mount several types of defense responses to block the pathogen completely or ameliorate the level of disease. Such responses include release of reactive oxygen species (ROS) and cell wall appositions (CWAs). A successful pathogen will have its own ROS detoxification mechanisms to manage this inhospitable environment. We are studying one such candidate mechanism in the rice blast fungus, *Magnaporthe oryzae*, governed by a gene we call *MoHYR1*, which encodes a glutathione peroxidase domain. Its yeast homolog is a thioredoxin-dependent peroxidase that forms disulfide bonds with a partner protein, YAP1, to regulate ROS detoxification. The wild type *MoHYR1* gene partially complemented the yeast mutant, but it was not rescued by the gene mutated in the cysteines that form bonds with YAP1. A *MoHYR1* deletion mutant showed growth inhibition in hydrogen peroxide (H2O2) and a decreased ability to break down plant-generated ROS during compatible interactions, including ROS associated with CWAs, resulting in significantly smaller lesions on barley and rice. Our results indicate that the *MoHYR1* gene functions similarly to its yeast homolog; it is important for fungal tolerance of H2O2, which is directly related to virulence.

Tipping the Balance: *Sclerotinia sclerotiorum* regulates autophagy, apoptosis and disease development by manipulating the host redox environment. Marty Dickman, Brett Williams, Mehdi Kabbage, and Hyo-Jin Kim. Institute for Plant Genomics and Biotechnology, Texas A&M University, Department of Plant Pathology and Microbiology, College Station, Texas 77843 USA

Disease symptoms in necrotrophic fungal infection have been attributed to direct killing of host tissue via secretion of toxic metabolites by the pathogen. Recently however, accumulating evidence from several pathosystems have suggested that such fungi are tactically more subtle in the manner by which pathogenic success is achieved, though the mechanistic details are not known. Sclerotinia sclerotiorum is a necrotrophic ascomycete fungus with an extremely broad host range (>400 species). This pathogen produces the non-specific phytotoxin and key pathogenicity factor, oxalic acid (OA). Our recent work indicated that the fungus and more specifically OA, can induce apoptotic-like programmed cell death (AP-PCD) in plant hosts. Importantly, we have also demonstrated that the induction of AP-PCD requires generation of ROS in the host, a process necessary for cell death and subsequent disease. Conversely, OA also dampens the plant oxidative burst, an early host response associated with defense. A challenge regarding OA in this context is the observation that OA both suppresses and induces host ROS during the same interaction. To address this issue, we have generated transgenic plants expressing a redox-regulated GFP reporter. Results show that initially, Sclerotinia (via OA) generates reducing conditions in host cells that suppress host defense responses including the oxidative burst and callose deposition, akin to hemi-biotrophic pathogens. Once infection is established however, Sclerotinia induces generation of plant reactive oxygen (ROS) leading to AP-PCD, of direct benefit to the pathogen. Chemical reduction of host cells with dithiothreitol (DTT) or potassium oxalate (KOA) restored the ability of the OA mutant to cause disease. Moreover the OA^- non-pathogenic, Sclerotinia mutants, induce autophagy in the host. Funding: NSF 0923918.

Scavenging of reactive oxygen species (ROS) as part of a hierarchical network of mitochondrial pathways involved in aging and lifespan control. Heinz D. Osiewacz[1], Andrea Hamann[1], Edda Klipp[2], Axel Kowald[2], Sandra Zintel[1]. [1]Johann Wolfgang Goethe University, Faculty of Biosciences and Cluster of Excellence Macromolecular Complexes, Frankfurt, Germany. [2]Humboldt University, Institute for Biology, Theoretical Biophysics, Berlin, Germany. E-Mail: Osiewacz@bio.uni-frankfurt.de

Biological aging is controlled by a complex mitochondrial network of interacting molecular pathways. Here we report the effect of a specific genetic manipulation of different components of the ROS scavenging system of the fungal aging model *Podospora anserina*. Unexpectedly, we found that the deletion of the gene coding for the mitochondrial manganese superoxide dismutase (PaSOD3) did not significantly affect the lifespan while over-expression let to severe impairments (e.g., growth rate, sensitivity against exogenous stressors) including a reduction in lifespan. Most strikingly, the up-regulation of only a single gene had a strong impact on the abundance of a number of proteins from different molecular pathways (e.g., ROS scavenging, proteolysis, heat-shock response) demonstrating the need for careful and systematic analyses of the effect of specific genetic manipulations. Such an analysis, which may utilize approaches of Systems Biology, is important to elucidate the impact and the interactions of individual pathways which in the past have been identified to contribute to biological aging.

Apoptotic fungal cell death mediates host invasion by pathogenic fungi. Amir Sharon, Neta Shlezinger, Sagi Shimshoni, Adi Doron. Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv 69978 Israel

The gray mold fungus *Botrytis cinerea* is a wide host-range plan pathogen causing significant crop losses worldwide. Most of the damage is due to post harvest rotting and infection of wounded plant tissues. Infection of intact plants is less significant, at least partly due to sensitive of the fungus to plant-derived anti-fungal compounds. During disease spreading, the fungus induces cell death in the host and inhabit only already killed tissues, thus avoiding direct contact with toxic plant metabolites. How the fungus mange to survive during the initial phase, when the spores are in contact with live host cells remains unclear. We hypothesized that apoptotic cell death (PCD) might be induced in the fungus by plant defense metabolites during the initial phase of disease establishment. Here we report on the characterization of PCD in *B. cinerea* and the role of the fungal anti-apoptotic response in disease development.
Bioinformatics searches revealed presence of a wide range of candidate apoptotic genes in fungi, but also absence of homologues of several important regulators of animal apoptosis. We isolated and characterized BcBir1, a *B. cinerea* homologue of IAP proteins, due to the central position of IAPs in mammalian apoptotic networks. Knockout or over expression strains of *BcBIR1* revealed that BcBir1 is anti-apoptotic and this activity was assigned to the N' terminal part of the protein. Using a strain expressing GFP-tagged nuclei and direct apoptosis assayed we found that the fungus undergoes massive programmed cell death during early stages of infection, but then fully recovers upon transition to second phase of infection. Further studies using the fungal mutants in combination with mutant *Arabidopsis* lines showed that fungal virulence was fully correlated ability of the fungus to cope with plant-induced PCD. Similar results were obtained with another necrotrophic pathogen *Cochliobolus heterostrophus.* Our result show that BcBir1 is major regulator of PCD in *B. cinerea* and that proper regulation of the host-induced PCD is essential for pathogenesis in this class of pathogens. Due to the general role of PCD in fungi and considering the common strategies of host invasion by pathogens, we propose that host-induced fungal PCD might be a general phenomenon including in human pathogens. The components of apoptotic networks, although only partially characterized, are conserved between fungi but differ from plant and animals. When considered together, it is expected that apoptotic networks might represent attractive targets for novel antifungal drugs.

Evidence that HxkC, an *Aspergillus nidulans* mitochondrial hexokinase-like protein, is anti-apoptotic. Margaret E. Katz[1], Rebecca Buckland[1], and Matthias Brock[2], [1]Molecular and Cellular Biology, University of New England, Armidale, NSW 2351 Australia, mkatz@une.edu.au [2]Microbiell Biochemistry, Hans-Knoell-Institut, Beutenbergstr. 11a, Jena 07745, Germany, Matthias.Brock@hki-jena.de

Binding of hexokinase II to mitochondria inhibits Bax-induced cytochrome *c* release from mitochondria and apoptosis in mammalian cells (Pastorino et al, 2002). HxkC, which plays a role in the response to nutrient stress, is the first fungal hexokinase shown to be associated with mitochondria (Bernardo et al. 2007). In a strain lacking functional HxkC, cleavage of DNA into oligonucleosomal fragments, a hallmark of mammalian apoptosis, occurs even in the absence of nutrient stress. This suggests that, as in plants, a fungal mitochondrial hexokinase inhibits programmed cell death even though Bax, a member of the Bcl-2 family, is not present. The *hxkC delta* null mutant shows increased susceptibility to oxidative stress but increased resistance to rapamycin-induced-inhibition of conidiation. Higher levels of intracellular protease activity, which could be the result of autophagy, are detected in the *hxkC delta* mutant. To determine whether HxkC plays a role in autophagy, we have generated mutants that lack both HxkC and AtgA, Although no loss of hexokinase activity was detected in the *hxkCdelta* mutant, purification of HxkC has revealed that the protein possesses low levels of ATPase and glucose-phosphorylating activity. Pastorino J.G., Shulga N., Hoek J.B. (2002) Mitochondrial binding of hexokinase II inhibits Bax-induced cytochrome *c* release and apoptosis. *J. Biol. Chem*. **277:** 7610-7618. Bernardo S.M.H., Gray K.-A., Todd R.B., Cheetham B.F., Katz M.E. (2007) Characterization of regulatory non-catalytic hexokinases in *Aspergillus nidulans. Mol. Genet. Genomics* **277:** 519-532.

Hyphal Tip Growth (Gow/Wendland) **Heather**

Tip growth in pollen tubes. An Yan, Gang Liu, Jamin Augusta, Guanshui Xu, and Zhenbiao Yang. Center for Plant Cell Biology, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521. yang@ucr.edu

The extreme polar growth, 'tip growth', is required for the formation of elongated tubular cells, which explore their environment or penetrate tissues, e.g., fungal hyphae invade animal and plant host tissues or explore nutrients in the environment, and pollen tubes penetrate female tissues to deliver sperms to the ovule for fertilization. Tip growth is achieved by targeting and fusion of vesicles to the apical region of the plasma membrane, termed tip growth domain. We investigate what defines and maintains the tip growth domain and how this domain directs growth using the pollen tube as a model system. Our previous studies suggest that the tip growth domain is generated and maintained by a self-organizing mechanism involving the ROP1 GTPase signaling network composed of an interlinking positive and negative feedback loops. Furthermore, our studies suggest that the ROP1 signaling network controls tip-targeted exocytosis. Using a combination of mathematical modeling and experimental approaches, we are testing the hypothesis that ROP1-dependent exocytosis is a central cellular event in pollen tube tip growth by coordinating the positive and negative feedback loops with the mechanical dynamics in the apical region of the cell wall.

Cytoskeleton and polarized growth. R. Fischer, N. Takeshita, C. Seidel, N. Zekert Karlsruhe Institute of Technology (KIT), Dept. of Microbiology, Hertzstr. 16, D-76228 Karlsruhe, reinhard.fischer@kit.edu

The interplay between the actin and the microtubule (MT) cytoskeleton in polarized growth of fungi has recently been revealed. In *S. pombe*, Tea1 – a so-called cell end marker protein - is transported to the plus ends of MTs by the kinesin Tea2, and is delivered to cell ends by hitchhiking with growing MTs. Mod5, which is posttranslationally modified by prenylation, anchors Tea1 at the cell ends, where Tea1 recruits formin for actin assembly. We showed recently that the functions are essentially conserved in *A. nidulans*. However, we found that the cell end marker complex is not only required for the polarization of the actin cytoskeleton but also for temporary attachment of the MTs to the complex. We also discovered that the correct localization of the cell end marker complex depends on sterol rich membrane domains. Several genes, involved in the formation of these membrane domains are currently studied. Recently, there is increasing evidence that endocytosis plays an important role in polarized growth. We characterized two Unc-104 related motor proteins and discovered that one of them, UncA, which is involved in endocytic vesicle transportation, preferentially moves along a detyrosinated MTs. Deletion analyses revealed a stretch of 80 amino acids in the tail of UncA important for the recognition of the special MT. To understand the function of different MT populations in A. nidulans, the ratio between tyrosinated and detyrosinated alpha-tubulin in the cell is modified by different means.

Roles of the Cdc42 polarity complex in hyphal tip steering. Emma Morrison[1], Stephen Milne[2], Neil Gow[1] and Alexandra Brand[1]. [1]Aberdeen Fungal Group, University of Aberdeen, UK. [2]Biosciences, University of Exeter, UK. (a.brand@abdn.ac.uk),

Fungal mycelia occupy a large variety of environmental niches and each species has a growth strategy that is optimised for its niche. A key effector in these strategies is the ability of hyphal tips to regulate their direction of growth in response to relevant environmental cues. These pre-programmed responses, or tropisms, are species-specific but are likely to be facilitated by the conserved molecular complexes that drive polarised hyphal growth. The hyphae of the human pathogen, *Candida albicans*, respond by re-orienting on contact (thigmotropism) and on the application of an electric field (galvanotropism). Using it as a model, we have shown that calcium is important for tropic growth responses and that hyphae are unable to respond appropriately to environmental cues when the GTP-cycling activity of the small GTPase, Rsr1/Bud1, is abolished, either by gene deletion or mutation. Rsr1 anchors the essential Cdc42 polarity complex correctly within the hyphal apex but the contribution of its interacting proteins in the tip-reorientation process is not known. We show that loss of GTP-cycling by Cdc42 severely reduces contact-sensing in *C. albicans* and causes hypha re-orientation in an electric field to become anodal instead of cathodal. This latter phenotype has also been observed by others in *Schizosaccharomyces pombe*, the fission yeast. To probe the role of calcium in the regulation of the polarity complex, we mutated the putative calcium-binding site in the Cdc42 activator, Cdc24, to disrupt its binding with the scaffold protein, Bem1. Interestingly, this mutant and the Bem1 reciprocal-binding mutant exhibited very different phenotypes, but both displayed a slightly heightened propensity to re-orient their tips compared to the control strain, the first time we have observed such an effect in a mutant. Hyphal tip response levels may reflect the stability of protein-protein interactions within the Cdc42 cell polarity complex.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Linking the Hgc1-Cdc28 CDK to the polarity machinery in *C. albicans* hyphal development. Yue Wang. Genes and Development Division, Institute of Molecular and Cell Biology, Singapore 138673

Cyclin-dependent kinases (CDKs) play a central role in yeast morphogenesis. Many years of studies in *Saccharomyces cerevisiae* have produced the following prevalent model. Cdk1 directs bud growth via association with different cyclins throughout the cell cycle: $Cdk1^{G1}$ promotes bud emergence and polarized growth at the bud tip, whereas $Cdk1^{G2}$ later switches the growth to isotropic expansion. Timing of the switch is critical: a premature switch produces round buds, whereas a delayed one causes bud elongation. Although well accepted and backed by strong evidence, direct links of CDKs with the cell's polarity machinery remain largely unknown; also the *S. cerevisiae* model has not been rigorously tested in other fungi. Using the dimorphic fungus *Candida albicans* as a model to study CDK's role in morphogenesis, we find that this organism has evolved a novel G1 cyclin, called Hgc1, specifically for promoting hyphal growth. Through a systematic screen for proteins that undergo CDK^{Hgc1}-dependent hyperphosphorylation, we have discovered several direct regulatory targets of CDK^{Hgc1} including the Cdc42 GAP Rga2, the septin Cdc11, the polarisome subunit Spa2, and the transcription factor Ace2. We also found that CDK^{Hgc1} associates with and negatively regulates the adenylyl cyclase Cyr1 to lower cellular cAMP levels, essential for maintaining hyphal growth. Together with findings by Peter Sudbery's and Haoping Liu's groups that Sec2 and Efg1 are CDK^{Hgc1}substrates respectively, our results have established concrete links between CDKs and cellular machines that drive key processes important for normal hyphal development.

Role of hyphal development in virulence of human fungal pathogen *Cryptococcus neoformans*. Marianna Feretzaki [1], Min Ni[1], Sarah H. Hardison[2], Floyd L. Wormley Jr. [2], and Joseph Heitman[1] [1]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, mf55@duke.edu [2]Department of Biology, University of Texas at San Antonio, San Antonio, Texas

Cryptococcus neoformans is a human fungal pathogen that causes lethal infections of the central nervous system in immunocompromised individuals. In the environment *Cryptococcus* has a defined sexual life cycle with **a** and alpha mating types. Mating leads to the formation of hyphae and spores that are considered to be the infectious propagules of the fungus. Following inhalation, the spores travel to the lung where they establish a pulmonary infection growing as budding yeast. Hyphal development inside the host is rare, possibly due to mammalian physiological conditions. However, our previous work has demonstrated that filamentation and key virulence factors, growth at high temperature and melanin synthesis, are governed by common genetic loci. In our study, we found that a hyperfilamentous strain is hypervirulent compared to the afilamentous, attenuated parental strain using murine and insect models. To further examine the progression of the infection, histological analysis showed that mice infected with the hyperfilamentous strain developed severe lung pathology with collapsed tissue and widespread growth compared to the attenuated parent. We performed linkage analysis to determine whether a single or multiple loci contribute to the observed difference in virulence. The progeny from a cross of the hyperfilamentous strain with the attenuated parent were screened for hyphal initiation and elongation, growth at high temperature and melanization. Murine and insect model hosts will be used to determine virulence of the interesting strains. Our ongoing studies focus on the mammalian innate immune response following infection of hyperfilamentous *C. neoformans*.

The signaling mucin Msb2 is processed into cellular and extracellular fragments during its function in appressorium formation of *Ustilago maydis*. Daniel Lanver, Patrick Berndt and Regine Kahmann. Max-Planck-Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl-von-Frisch-Strasse 10, D-35043 Marburg, Germany. daniel.lanver@mpi-marburg.mpg.de

The dimorphic fungus *Ustilago maydis* switches from budding to hyphal growth on the plant surface. In response to hydrophobicity and hydroxy fatty acids *U. maydis* develops infection structures called appressoria. Here we report on the transmembrane mucin Msb2, which is essential for appressorium formation in response to the hydrophobic stimulus. The Msb2-protein is processed into a cellular fragment and a large, highly glycosylated extracellular part. The latter fragment remains attached to the cell surface, as demonstrated by immunofluorescence and western analysis, but is also shed from cells. Although deletion of either part of Msb2 does not alter localization of the remaining part, both, the extracellular and intracellular domains are essential for function. We propose that the cellular part of Msb2 plays a role in activating downstream signaling events. Epistasis analysis revealed that Msb2 acts upstream of Kpp2 and Kpp6, two MAP-kinases essential for plant cuticle penetration. Collectively, our data indicates that Msb2 in *U. maydis* is a plasma membrane receptor involved in plant surface sensing. To reveal the impact of the Msb2-protein on gene regulation we performed genome-wide transcriptional profiling at the stage of appressorium formation and will discuss these results.

Intercalary extension in vegetative hyphae facilitates colonisation of developing grass leaves by fungal endophytes. Christine R. Voisey[1], Suzanne J.H. Kuijt[1], Mike J. Christensen[1], Wayne R. Simpson[1], Kelly Dunstan[1], K.G. Sameera U. Ariyawansa[1], Nick D. Read[2], Neil A.R. Gow[3], Rosie E. Bradshaw[4], Hironori Koga[5]and Richard D. Johnson[1]. [1]AgResearch, Palmerston North, New Zealand.[2]University of Edinburgh, Edinburgh, Scotland.[3]University of Aberdeen, Aberdeen, Scotland.[4]Massey University, Palmerston North, New Zealand.[5]Ishikawa Prefectural University, Nonoichi, Ishikawa, Japan. christine.voisey@agresearch.co.nz

The highly polarised process of apical extension in vegetative hyphae is a distinguishing characteristic of fungal growth. However, an exception to this paradigm has recently been observed in endosymbiotic fungi that infect temperate grasses from seed. Grasses in the sub-family *Pooideae* form symbiotic associations with endophytic fungi of the genera *Epichloë* and *Neotyphodium*. The fungi colonise aerial tissues of developing grass seedlings by infecting the primordia of leaves and inflorescences as they develop on the shoot apical meristem. The hyphae of the endophyte are firmly attached to growing plant cells, and the two organisms are therefore committed to undertake coordinated developmental programmes.The leaves of grasses grow primarily though intercalary extension, a result of significant cell expansion throughout the leaf expansion zone. Conversely, vegetative fungal hyphae are thought to grow exclusively at the hyphal apex. In a striking example of co-evolution, these fungi have evolved a novel mechanism of elongation and division in intercalary compartments. This extremely rare mode of growth has enabled the attached endophyte to grow in synchrony with the host. The molecular and cytological events that orchestrate cell wall extension are the subject of a new study aimed at determining the specific orientation of the cytoskeleton and the movement of chitomes during polar and intercalary modes of growth. Two key fungal signalling pathways are currently being investigated to establish whether they participate in coordinating responses to mechanical stress which may trigger intercalary growth in endophytes when host cells expand.

Polarity proteins Bem1 and Cdc24 are components of the filamentous fungal NADPH oxidase complex. Daigo Takemoto[1,2], Sachiko Kamakura[3], Sanjay Saikia[2], Yvonne Becker[2], Ruth Wrenn[2], Aiko Tanaka[1,2], Hideki Sumimoto[3] and Barry Scott[2] [1] Graduate School of Biogricultural Sciences, Nagoya University, Nagoya, Japan. [2] Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand. [3] Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Endophytic fungi *Epichloë festucae* systemically colonize the intercellular spaces of perennial ryegrass to establish a symbiotic association. We have shown that reactive oxygen species (ROS) produced by a specific NADPH oxidase isoform, NoxA, a regulatory component, NoxR, and the small GTPase, RacA have a critical role in regulating hyphal growth in the host plant. Generation of ROS by *E. festucae*, requires functional assembly of a multi-subunit complex composed of NoxA, NoxR, and RacA. However, the mechanism for assembly and activation of this complex at the plasma membrane is unknown. We found that *E. festucae* NoxR interacts with homologs of the yeast polarity proteins, Bem1 and Cdc24, and that the PB1 protein domains found in these proteins are essential for these interactions. GFP fusions of BemA, Cdc24 and NoxR preferentially localized to actively growing hyphal tips and to septa. These proteins preferentially interact with each other *in vivo* at these same cellular sites as shown by bimolecular fluorescent complementation assays. The PB1 domain of NoxR is essential for localization to the hyphal tip. An *E. festucae bemA* mutant was defective in hyphal morphogenesis and growth in culture and *in planta*. The changes in fungal growth *in planta* resulted in a defective symbiotic interaction phenotype. These results demonstrate that BemA and Cdc24 play a critical role in localizing Nox proteins to sites of fungal hyphal morphogenesis and growth.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Abstracts for Posters

Comparative and Functional Genomics

1. Peculiar chromosomal evolutionary processes in filamentous fungi, RIP, lateral gene transfer, sectional gene loss and mesosynteny, are amplified in dothideomycetes. Richard P. Oliver, James K Hane, Robert Syme, ACNFP, Curtin University, Australia, Richard.oliver@curtin.edu.au

Comparisons of fungal genomes have highlighted four processes which seem to be prevalent and even unique in filamentous fungi. The best known is RIP, a genome defence mechanism that mutates copies of repeated sequences during meiosis. We have developed semi-automated methods of detecting RIP in genome assemblies and of predicting the progenitor sequences. The acquisition of genome sequences of multiple isolates of , has revealed patterns of gene conservation within a species. Extraordinary numbers of genes are unique to each isolate. Furthermore the distribution of "missing" genes is not random. Rather many runs of adjacent genes are missing - a phenomenom we call sectional gene loss. Thirdly comparison of gene content between species suggests that many genes have been laterally transferred into and between filamentous species on times scale that range from decades to millions of years. Comparisons between species reveals a novel form of synteny characterised by retained chromosomal gene content, but shuffled gene order and orientation; we call this mesosynteny. Mesosynteny promises to expedite genome finishing. These four processes are prevalent with the Dothideomycetes, a group that includes the most damaging plant pathogens. We will discuss whether these phenomena have causal relationships and whether this group's success as agricultural patterns can be attributed to these peculiar evolutionary processes.

2. An *in vivo* transcriptome for the entomopathogenic fungus *Metarhizium robertsii* ARSEF 2575. B. Giuliano Garisto Donzelli[1], B. A. Roe[2], · S. L. Macmil[2], D. J. Schneider[1], G. DeClerck[1], A. C. L. Churchill[3], D. M. Gibson[1]. [1] R. W. Holley Center, USDA-ARS, Tower Road, Ithaca, New York 14853. [2]Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma, 73019. [3]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, New York, 14853.

To evaluate the transcriptional responses of the insect pathogen *Metarhizium robertsii* ARSEF 2575 during its interaction with insects, we developed a method to specifically recover pathogen transcripts from infected *Spodoptera exigua* (beet armyworm) larvae. cDNA was sequenced using the Roche 454 GS-FLX platform and the resulting 344,678 reads were attributed to either the pathogen or the host based on similarity searches against the GenBank nr and est_others databases. Out of 34,652 unigenes,14,298 had significant similarity to known fungal proteins or transcripts; 2,748 unigenes were attributable to the host. All of the *M. robertsii* virulence factors described to date were represented, alongside with several novel transcripts potentially involved in virulence, including cell wall proteins, iron homeostasis genes and hydrolytic enzymes. On the host side, the library contained mainly transcripts of housekeeping genes, only ~50 of which were associated with immune defense responses. A more complete annotation of the library based on the *M. robertsii* ARSEF 2575 genome is in progress.

3. Mapping the Hsp90 Chaperone Network in *Candida albicans* Reveals Environmental Contingency and Rewired Circuitry. Stephanie Diezmann*, Magali Michaut˚, Gary Bader˚, Leah E. Cowen* * Department of Molecular Genetics, University of Toronto, 1 King's College Circle, Toronto ON M5S 1A8 ˚ Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, 160 College Street, Toronto ON M5S 3E1

Hsp90 is an essential molecular chaperone conserved in all eukaryotes. It regulates the stability of diverse client proteins, including many signal transducers. In the leading fungal pathogen of humans, *Candida albicans*, Hsp90 governs cellular circuitry regulating fungal drug resistance and morphogenesis, though only two client proteins have been identified to date. We mapped the *C. albicans* Hsp90 genetic interaction network and followed its dynamics under different stress conditions, such as high temperature, high osmolarity, and exposure to drugs that compromise the cell wall and cell membrane. We conducted a chemical genetic screen with a library comprising ~1,200 mutants covering 10% of the genome and the Hsp90 inhibitor geldanamycin, and identified a total of 226 interactions. Network analyses revealed common and unique stress response profiles. Further characterization of specific interactors suggests that some of them act upstream to modify Hsp90 function while others are downstream and depend on Hsp90 for activation. A comparison with *Saccharomyces cerevisiae* revealed a largely distinct *C. albicans* Hsp90 interaction network, suggesting rewiring of the network probably in response to different niche requirements and the selective pressure exerted by different life histories.

4. Genome sequence analysis of *Chrysosporium lucknowense* C1: a filamentous fungus of biotechnological importance. Hans Visser[1], Sandra Hinz[1], Martijn Koetsier[1], Vivi Joosten[1], Scooter Willis[2], Bruce Pascal[2], and Jan Wery[1]. [1]Dyadic Netherlands, Wageningen, Netherlands. [2]Scripps Institute, Jupiter, Florida, USA.

The ascomycetous fungus *Chrysosporium lucknowense* C1 was developed as an efficient and versatile platform for high level protein production on a commercial scale, providing a strong alternative to well established industrial fungi, like *Aspergillus niger* and *Trichoderma reesei*. Strain and process improvement strategies of the original C1 isolate resulted in strains that are able to secrete large amounts of a complex mixture of (hemi-)cellulases. Re-sequencing and automated annotation of the wild type C1-genome using the latest sequencing and bioinformatics tools revealed that C1 is a rich source of (potential) industrial enzymes. These enzymes include oxido-reductases, proteases, esterases and hydrolases. In particular, the approximately 38 Mbp genome appeared to be very rich in genes encoding plant biomass hydrolyzing enzymes. Comparison of this plant cell wall degrading capacity with that of *A. niger* and *T. reesei* revealed interesting similarities and differences. C1 specifically differentiates itself from *A. niger* and *T. reesei* by the relatively large number of (glucurono-) arabinoxylan degrading enzymes. However, C1 and *A. niger* are similar with respect to the number of cellulases, while *T. reesei* has notably less cellulases. An overview of these and other C1 genome sequence data as well as some examples of the exploitation thereof will be presented.

5. Correlation of gene expression and protein production rate - a system wide study. Mikko Arvas, Tiina Pakula, Bart Smit, Jari Rautio, Paula Jouhten, Heini Koivistoinen, Erno Lindfors, Marilyn Wiebe, Merja Penttila and Markku Saloheimo VTT Technical research centre of Finland, Tietotie 2, 02044 VTT, Finland mikko.arvas@vtt.fi

The filamentous fungi *Trichoderma reesei* is an industrial protein production host with exceptional protein secretion capability. It can produce up 100 g/l of protein and it is often used to produce cellulases and hemicellulases to depolymerise biomass. Most efficient production of secreted protein is achieved at low specific growth rate of 0.03 1/h (Pakula et al. Microbiology 151 (2005), 135-143). We have used transcriptomics and proteomics to study the effect of growth rate and cell density to *T. reesei* protein production in chemostat cultivations. Use of chemostat allows control of growth rate and precise estimation of the specific protein production rate (SPPR). For each gene we calculate its correlation to the SPPR and analyse the distribution of these correlations in context of genome structure and annotation, gene sequence homology to other fungi and predicted metabolic network. Although highest protein production occurs at low growth rate and carbon limitation the observed response is distinct from *Saccharomyces cerevisiae*'s response to low growth rate or carbon limitation. In addition to conventional transcriptomics by microarray, the used microarray also included probes for non-coding sequence to detect novel genes (Arvas et al. Gene 467 (2010) 41-51). Several novel genes, putatively related to regulation, were discovered as differentially expressed in these conditions.

6. Lascer capture microdissection, RNA-seq, and mutant genome sequencing: How to use next-generation sequencing to characterize developmental genes in filamentous fungi. Minou Nowrousian, Ines Teichert, Ulrich Kück Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Bochum, Germany; email minou.nowrousian@rub.de

Next-generation sequencing (NGS) techniques have revolutionized the field of genomics/functional genomics. We have recently sequenced and assembled the genome of the filamentous ascomycete *Sordaria macrospora*, a model organism for fungal development, solely from NGS reads (PLoS Genet 6:e1000891). We are now applying NGS in two approaches for the identification and characterization of developmental genes. (I) With laser capture microdissection, we can separate protoperithecia from the surrounding hyphae. RNA isolation and amplification from 150 protoperithecia yields enough material for RNA-seq analysis. The resulting data will be compared to RNA-seq data from whole mycelial exctracts to characterize the genome-wide spatial distribution of gene expression during sexual development. (II) We sequenced the genomes from two mutants that were generated by conventional mutagenesis, and identified two causative mutations through bioinformatics analysis. One mutant carries a mutation in the known developmental gene *pro41*. The second, a spore color mutant, has a point mutation in a gene that encodes an enzyme of the melanin biosynthesis pathway. For both mutants, transformation with a wild-type copy of the affected gene restored the wild-type phenotype. These data show that whole genome-sequencing of mutant strains is a rapid method for the identification of developmental genes.

7. The genome sequence for *Eremothecium cymbalariae* establishes a link between the *S. cerevisiae* ancestor and the streamlined genome of *Ashbya gossypii*. Juergen Wendland and Andrea Walther, Carlsberg Laboratory, Yeast Biology, DK-2500 Valby, Copenhagen, Denmark; jww@crc.dk

E. cymbalariae is a close relative of *A. gossypii*. Both species are filamentous fungi that show bifurcational (Y-shaped) tip growth. In contrast to *A. gossypii*, *E. cymbalariae* generates an aerial mycelium with hyphae that form sporangia at their tips. *E. cymbalariae* spores lack appendices with which spores of *A. gossypii* stick together in bundles. To explore these differences on a genomic level we have established the complete genome sequence for *E. cymbalariae* using a 454 approach. We obtained a 40x coverage of the genome and with additional paired-end sequencing of fosmids and directed PCRs assembled the genome of app 9.6Mb into *E. cymbalariae's* 8 chromosomes in contrast to only 7 chromosomes in *A. gossypii*. We found orthologs of app. 4700 genes present in the yeast ancestor plus app 170 tRNAs. Most of the genes of *E. cymbalariae* are within blocks of synteny with the yeast ancestor. Strikingly the conservation of synteny is greater between *E. cymbalariae* and the ancestral yeast rather than to *A. gossypii*. At syntenic positions several homolgs to *S. cerevisiae* or e.g. *K. lactis* are present in the *E. cymbalariae* genome that are absent from *A. gossypii*. This indicates that the *E. cymbalariae* genome represents a preWGD genome with close ties to the ancestral yeast. During evolution several decisive changes occurred in the *A. gossypii* genome that affected for example the mating-type loci, the removal of a transposon, the condensation of intergenic regions, a strong increase in GC-content, and chromosomal rearrangements. We will present phenotypic comparisons of *E. cymbalariae* and *A. gossypii* as well as insights into genome evolution of the *Eremothecium* lineage.

8. Comparative Genomics Suggests the Presence of RNA Interference in Oomycetes. Nahill Matari and Jaime E. Blair Department of Biology, Franklin and Marshall College, Lancaster, PA

RNA interference is a natural process eukaryotes use to regulate gene expression. Here we used comparative genomic approaches to identify the genes involved in RNA interference within available Oomycete genomes. Amino acid sequences of proteins known to be involved in RNAi biogenesis from human, *Drosophila*, and *Arabidopsis* were collected and used as references to search Oomycete genomes for the presence of homologs. Dicer, drosha, argonaute, and pasha protein sequences were used as queries as they are known to be crucial for RNAi biogenesis and are heavily conserved between different organisms. Searches yielded that *Phytophthora ramorum, P.capsici, P.infestans, P.sojae*, and *Saprolgenia parasitica*, as well as outgroups *Thalassiosira pseudonana* (diatom) and *Ectocarpus siliculosus* (brown alga), all contain proteins that are homologous to the reference sequences. Pfam was used to verify that each homolog contained the appropriate protein domains known to be involved in RNAi biogenesis. Phylogenetic analysis of both protein and nucleotide data suggest that these genes have also experienced multiple rounds of duplication within Oomycetes. These results suggest that Oomycete genomes contain the appropriate genes necessary for RNA interference. Currently, nucleotide alignments are being used to design primers for both genomic PCR and RT-PCR for test for the presence and expression of these genes in locally collected isolates of *Phytophthora* and *Pythium*.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

9. *sex* gene in *Rhizopus oryzae.* Andrii Gryganskyi[1], Soo Chan Lee[1], Anastasia Litvintseva[1], O. Savitskyi[2], Iryna Anishchenko[2], Josef Heitman[1] and Rytas Vilgalys[1]. [1]Duke University, Durham, USA. [2]Ukrainian Academy of Sciences, Kyiv, Ukraine.

The *Rhizopus oryzae* species complex comprises a group of zygomycetous fungi that are common, cosmopolitan saprotrophs. Some strains are used for production of tempeh and other Asian fermented foods but they can also act as opportunistic human pathogens. Although *R. oryzae* reportedly has a heterothallic (+/-) mating system, most strains are incapable of sexual reproduction, and the genetic structure of its mating locus has not been characterized. Here we report on the mating behavior and genetic structure of the mating locus from 57 isolates of the *R. oryzae* complex, including the recently sequenced genome of isolate RA99-880. All strains from the *R. oryzae* complex have a mating locus that is similar in overall organization to the mucoralean fungi *Phycomyces blakesleeanus* and *Mucor circinelloides*. In all of these fungi, the minus allele features a high mobility gene (HMG) flanked by an RNA helicase gene and a triose phosphate transporter gene. Within the *R. oryzae* complex, the plus mating allele includes a large inserted region that codes for a BTB/POZ domain gene. Phylogenetic analysis of HMGs, ITS and 28s rDNA, RPB2, mtSSU and LDH genes in *R. oryzae* isolates identified two distinct groups that correspond to previously described sibling species (*R. oryzae* s.s. and *R. delemar*). Laboratory mating assays identified a phenotypic difference between these species.

10. Utilizing functional genomics in *Neurospora crassa* to identify cell wall genes. Divya Sain and Jason E. Stajich, University of California, Riverside, CA dsain001@ucr.edu

The cell wall is one of the most important organelles of the fungal cell and differentiates the pathogenic fungi from the plants and animals they infect. This makes the cell wall an excellent target for anti-fungal drugs. We employed a functional genomics approach to identify additional cell wall genes in the model filamentous fungus, *Neurospora crassa*. While models of the cell wall biosynthesis were elucidated in the model yeast *Saccharomyces cerevisiae*, filamentous fungi may have additional genes and pathways. Using genetic and genomic tools to investigate cell wall mutant phenotypes in *N. crassa* will provide insight into pathways needed for walls and growth in filamentous fungi. It has been shown that the growing fungal hyphal tip has high cell wall biosynthesis activity (Bartnicki-Garcia and Lippman, Science, 1969) and a recent microarray study of *N. crassa* hyphal growth at 6 timepoints (Kasuga and Glass, Euk Cell, 2008) showed that the genes expressed at the tips of the colony were enriched for cell wall functions. We mined the microarray results and found 60 genes were highly expressed in the tip. These included known cell wall genes like CHS (chitin synthase) and FKS1 (1,3-beta glucan synthase); while many others have no known function. We screened knock- out mutants of these genes for basic growth, developmental abnormalities, and defects in the cell wall integrity pathways. The mutants showing abnormal phenotypes will serve as a starting set for future anti-fungal screening and additional genetic analysis.

11. Characterization of Class II DNA Transposons in *Coprinopsis cinerea.* Sydney Webb and Marilee A. Ramesh. Department of Biology, Roanoke College, Salem, VA 24153. ramesh@roanoke.edu

An analysis of the *Coprinopsis cinerea* genome has identified three families of DNA transposons (Class II repetitive elements). While both Class I and Class II repetitive elements were found in the *C. cinerea* genome, Class II elements appear to occur at a much lower frequency. Bioinformatics techniques were used to analyze and characterize three main families of Class II elements within *C. cinerea*. The *C. cinerea* genome assembly and annotation was searched with 150 pFAM domains specific for eukaryotic transposons, identifying three families of elements. One family, the En/Spm family, contains 12 transposons ranging in size of 4044 to 3468 bp. This family is closely related to the CACTA superfamily in plants, although it does not appear to contain the conserved terminal regions seen in the plant elements. To further characterize the En/Spm transposons, the exon and intron lengths were assessed. The upstream and downstream regions were analyzed to identify and characterize each element's Terminal Inverted Repeat (TIR) region. Potential TIR repeats of 3-6 bp sequences have been identified on the upstream and downstream regions. A similar approach is being taken to analyze the other two families of Class II elements (hAT and mariner) in the *C. cinerea* genome and preliminary data will be presented.

12. Mating-type loci in the homothallic Ascomycete *Eupenicillium crustaceum*. Stefanie Pöggeler[1], Céline M. O'Gorman[2], Birgit Hoff[2] and Ulrich Kück[2] [1] Department of Genetics of Eukaryotic Micro-organisms, Institute of Microbiology and Genetics, Georg- August University, Grisebachstr. 8, 37077 Göttingen, Germany; e-mail: spoegge@gwdg.de [2]Department of General and Molecular Botany, Ruhr-University Bochum, Universitätsstraße 150, 44780 Bochum, Germany

The homothallic *Eupenicillium crustaceum* Ludwig is very closely related to the penicillin-producer *Penicillium chrysogenum*, which is supposed to reproduce only asexually. However, recently strains of *P. chrysogenum* have been shown to carry either the mating type (*MAT*) locus *MAT1-1* or *MAT1-2* suggesting a heterothallic breeding system. To analyze the molecular basis of homothallism in E. crustaceum, we cloned and sequenced its *MAT* sequences. Two *MAT* loci, *MAT1-1* and *MAT1-2*, reside in the genome of *E. crustaceum. MAT1-1* is flanked by conserved *apn2* and *sla2* genes and encodes a homologue of the alpha-box domain protein MAT1-1-1, while *MAT1-2* carries the HMG domain gene *MAT1-2- 1* and is flanked by a degenerated *sla2* gene and an intact homologue of the *P. chrysogenum* ORF *Pc20g08960*. To determine functionality of the *E. crustaceum MAT* genes, we demonstrate their transcriptional expression during vegetative development. Furthermore, the alpha-box domain sequence of MAT1-1-1 and the HMG domain sequence of MAT1-2-1 were used to determine the phylogenetic relationship with other ascomycetes. Phylogenetic trees confirmed strong relationships between the homothallic *E. crustaceum* and the supposedly heterothallic *P. chrysogenum*.

13. A functional genomics study of extracellular protease production by *Aspergillus niger*. Machtelt Braaksma[1], Mariet van der Werf[1], Cees van den Hondel[1,2] and Peter Punt[1,2] 1, TNO Microbiology and Systems Biology, Zeist , the Netherlands 2, Molecular Biology and Biotechnology, Leiden University

To study the complex regulation of extracellular proteases in the filamentous fungus *Aspergillus niger* a combination of comprehensive functional genomics technologies was used. The requirements for performing a successful systems biology study and addressing the challenges met in analyzing the large, information-rich data sets is adressed. Protease activity plays an important role in strain and process development of *A. niger* and other aspergilli. In our research the influence of several environmental factors on the production of extracellular proteases in controlled batch cultivations was studied. Samples generated in this study were used for analysis with functional genomics technologies. With a shotgun proteomics approach the *A. niger* secretome under different experimental conditions was determined. Furthermore, the effect of different quantitative phenotypes related to protease or glucoamylase activity on the information content of a metabolomics data set was investigated. Based on a transcriptomics study the identification of co-expression networks is described. First, a set of conserved genes was used to construct these networks. Subsequently, all annotated genes, including hypothetical and poorly conserved genes, were integrated into the co- expression analysis, allowing identification of novel targets for strain and process development.

14. A Software System for Analysis of Next Generation Sequencing Data of Fungal Strains. Asa Oudes[1], Thomas Hartsch[2], Sebastien Ribrioux[2], Nadim Jessani[1], & Hans-Peter Fischer[2] Genedata, Inc., San Francisco, USA[1], Genedata AG, Basel, Switzerland[2]

Next generation sequencing (NGS) has greatly increased the amount of data generated in genomic studies. NGS applications such as genome and transcriptome sequencing are approaches that produce large volumes of data. However, a generic integrative approach for management, visualization and analysis of NGS data does not exist. In close collaboration with our customers we developed a flexible, scalable and integrative software solution called Genedata Selector to provide a platform for genomic studies. The Selector system facilitates integration of public and proprietary data in one database and contains built in tools for data analysis and visualization. With a software system such as Selector, studies that generate vast volumes of genomic data which in the past presented a daunting data management and analysis task, are now approachable. We will illustrate how standard genomics technologies together with NGS data of fungi can be used to elucidate the genetic variation, metabolic capacities, and stability of strains at a genome wide level. The Selector system supports cross-omics data analysis and we will show how diverse experimental data can be used to generate a comprehensive analysis of fungal genomes. The analysis tools we will demonstrate are useful in basic research and industrial biotechnology applications such as bio-fuels, enzyme production, and feed or food ingredient production.

15. Mycotoxin gene expression in response to temperature in *Aspergillus flavus*. Jiujiang Yu[1], Natalie Fedorova[2], Beverly G. Montalbano[1], Deepak Bhatnagar[1], Thomas E. Cleveland[1], Joan W. Bennett[3], William C. Nierman[2] [1]USDA/ARS/SRRC, New Orleans, LA. [2]The J Craig Venter Institute, Rockville, MD. [3]Rutgers University, New Brunswick, NJ.
We investigated the transcriptome by RNA-Seq in *Aspergillus flavus* under temperature of conducive to aflatoxin production (30C) vs. not conducive condition. For each sample, over 10 million reads were generated and mapped to over 70% known *A. flavus* genes. Further analysis revealed quantitative differences in gene expression between the conditions tested. It demonstrated that aflatoxin production is one of the most tightly regulated processes in a fungal cell. The transcript abundance for aflatoxin biosynthesis genes was 1000 times greater under conditions conducive to aflatoxin production. The data defined the complete aflatoxin biosynthesis gene cluster consisting of 30 genes. Our results are consistent with the view that high temperature negatively affects aflatoxin production by turning down transcription of the two key regulators, aflR and aflS. It affects more on *aflS* than *aflR*. In addition, over 500 other genes were differentially expressed under the conditions tested. Remarkably, the RNA-Seq approach also exposed thousands of transcripts that have not been previously identified. These included novel protein-coding and non-coding genes, which are being further characterized. This study shows that the RNA-Seq technology can provide an unprecedented high resolution view of the transcriptome and reveal additional transcript complexity.

16. Comparative Analysis of Thermophillic Fungal Genomes. Robert Otillar, Asaf Salamov, Frank Korzeniewski, Jeremy Schmutz, Erika Lindquist, Adrian Tsang, Randy Berka, Igor Grigoriev

Rapid, efficient, and robust enzymatic degradation of biomass-derived polymers is currently a major obstacle in biofuel production. A key missing component in that process is the availability of enzymes that hydrolyze cellulose, hemicellulose, and other polysaccharides into biofuel substrates at temperatures and chemical conditions suitable for industrial use. Thermophilic fungi are known to excrete enzymes that rapidly degrade polymers including cellulose and hemicellulose at high temperatures, however the genome sequence and full gene complement of thermophillic fungi have not been previously reported. Here we describe the initial sequencing, gene identification, and comparative analysis of two thermophilic fungi, *Thielavia terrestris* and *Sporotrichum thermophile*, with comparison to *Chaetomium globosum*, a closely related non-thermophillic fungal species.

17. Evolutionary genomic analysis of fungal Cytochrome P450 proteins with Fungal Cytochrome P450 Database 1.2. Venkatesh Moktali[1], Jongsun Park[2], Yong-Hwan Lee[2], Seogchan Kang[1]. [1]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA. [2]Fungal Bioinformatics Laboratory, Seoul National University, Seoul 151-921, Korea. Venkatesh Moktali: vpm104@psu.edu Prof. Seogchan Kang: sxk55@psu.edu

Cytochrome P450 proteins (CYPs) play diverse and pivotal roles in fungal biology and ecology. Their ability to detoxify harmful environmental chemicals and involvement in the production of many secondary metabolites underpins the adaptation of fungi to specific hosts or environments. The rapid increase of sequenced fungal genomes representing diverse taxa has enabled large-scale phylogenomic studies to investigate the evolutionary mechanisms of the genes encoding CYPs in the fungal kingdom. Comparison of CYPs across species has shown signs of gene birth and death, suggesting that understanding of how and when such events occurred likely provides new insights into fungal evolution. The rapid increase in the number of CYPs identified through genome sequencing also poses a major challenge in classifying CYPs systematically. The poster describes a method to cluster CYPs using the Tribe-MCL algorithm. We have also reviewed the function of previously characterized CYPs and analyzed the patterns of evolution in this protein family based on a recently established web platform, the Fungal Cytochrome P450 Database (http://p450.riceblast.snu.ac.kr/index.php). The results of these analyses will also be presented in the poster.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

18. Comparative genomics of *Coccidioides* and its close relatives. Emily Whiston[1] and John Taylor. U.C. Berkeley, Berkeley CA. [1] whiston@berkeley.edu

The mammalian pathogens *Coccidioides immitis* and *C. posadasii* are the only dimorphic fungal pathogens that form spherules in the host. Furthermore, all of *Coccidioides*' closest known relatives are non-pathogenic. In this project, we are interested in genome changes between the *Coccidioides* lineage and its relatives. In the last few years, full genomes have become available for *Uncinocarpus reesii* and 20 *Coccidioides* species; *Coccidioides* and *U. reesii* are estimated to have diverged 75-80 million years ago. Here, we have sequenced the genomes of 4 species more closely related to *Coccidioides* than *U. reesii*: *Byssoonygena ceratinophila*, *Chrysosporium queenslandicum*, *Amauroascus niger* and *A. mutatus*. For each of these 4 species: we isolated DNA, prepared libraries for Illumina sequencing and generated 3 lanes of 101bp paired-end sequence data. Genomes were assembled using the SOAP de novo sequence assembly pipeline (Corrector, SOAPdenovo and GapCloser). The 4 assembled genomes ranged from 23-34Mb, with N50 of 90kb-205kb. Preliminary gene calls were generated using SNAP. With this data, we will report on evidence of positive selection, individual gene gain/loss, and gene family expansion/contraction.

19. A new mutant phenotype system and the curation of pathogenesis-related phenotypes for *Aspergillus nidulans* and *Aspergillus fumigatus* at the *Aspergillus* Genome Database. Diane O. Inglis [1], Martha B. Arnaud [1], Jon Binkley [1], Gustavo Cerqueira [2], Maria C. Costanzo [1], Marcus C. Chibucos [2], Jonathan Crabtree [2], Joshua Orvis [2], Prachi Shah [1], Marek S. Skrzypek [1], Gail Binkley [1], Stuart R. Miyasato [1], Jennifer R. Wortman [2] and Gavin Sherlock [1] [1] Department of Genetics, Stanford University School of Medicine, Stanford, CA [2]Institute for Genomic Sciences, University of Maryland School of Medicine, Baltimore MD

The *Aspergillus Genome Database* (AspGD; www.aspgd.org) collects and displays gene, protein, and genomic information gathered from published literature about the model fungus, *Aspergillus nidulans* and other aspergilli including the pathogen *A. fumigatus*. Much of the curated information in AspGD is described using controlled vocabularies, such as the Gene Ontology, which greatly facilitates searching for specific data and comparison across genomes. We use a new phenotype curation system that conforms to a rigorously controlled-vocabulary system that was developed at the *Saccharomyces* Genome Database (SGD). Each phenotype annotation is broken down into an "observable", representing the entity or process that is observed (e.g., conidiation) and a qualifier that describes the effect on that entity or process in the mutant (e.g., decreased, increased, abnormal, normal). Additional fields may contain information about the mutant such as strain background, allele name, conditions under which the phenotype is observed or the animal model used in a virulence assay. To more comprehensively capture host-pathogen interaction phenotypes, we have expanded our phenotype vocabulary to include the additional pathogenesis-related terms, "resistance to killing by host cells" and "resistance to phagocytosis" in addition to terms, such as virulence, that were already in use. A summary of the mutant phenotype information is displayed on the Locus Summary page for each gene, and the complete information is displayed in tabular format on the Phenotype details page for each gene. All of the information is searchable, and may also be downloaded in bulk using AspGD's Batch Download tool or from the Download Data page. AspGD is supported by grant RO1 AI077599 from the NIAID at the NIH.

20. Identification of new nonself recognition loci in *Neurospora crassa.* Elizabeth Hutchison, Charles Hall, and N. Louise Glass. Plant and Microbial Biology Department, University of California, Berkeley, CA 94720-3102. hutchison@berkeley.edu, charleshall@berkeley.edu, lglass@berkeley.edu

Nonself recognition mechanisms are prevalent across a diverse array of organisms. The filamentous ascomycete *Neurospora crassa* can undergo hyphal fusion to form heterokaryons, however, *N. crassa* employs a nonself recognition mechanism termed heterokaryon incompatibility (HI) to restrict hyphal fusion to genetically identical strains. Fusion cells between strains different at any one of eleven heterokaryon (*het*) loci are rapidly compartmentalized and undergo programmed cell death. Interestingly, almost all molecularly characterized *het* loci are associated with a gene containing a HET protein domain; HET domains are specific to filamentous ascomycete species, and over- expression of just the HET domain can cause cell death. *N. crassa* has approximately 50 HET domain genes, but it is unlikely that all are involved in HI. Previous evolutionary analysis of *het* loci revealed that they are highly polymorphic, and that allele classes are maintained by balancing selection. Thus, in order to identify HET domain genes that function in HI, we identified polymorphic HET domain genes showing signatures of balancing selection using a comparative genomics approach and an RNA-Seq dataset from ~120 wild *N. crassa* isolates. We identified approximately 20 candidate polymorphic HET domain genes. One of the candidate genes, NCU09037, has two distinct alleles in an *N. crassa* population, and these alleles exhibit balancing selection.

21. Mitotic chromosomes and karyotype of *Colletotrichum* orbiculare. Masatoki Taga[1], Kaoru Tanaka[2] and Yasuyuki Kubo[2]. [1]Department of Biology, Okayama University, Okayama 700-8530, Japan; [2]Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan

Colletotrichum orbiculare (syn. *C. lagenarium*) is an ascomycete causing anthracnose in cucumber. Although it has served as a model organism to study appressorium-aided invasion of fungal pathogens into plants, almost no information was available of its genome. In this study, we analyzed mitotic chromosomes and karyotype of *C. orbiculare* using a standard wild type strain 104-T (MAFF240422). Pulsed field gel electrophoresis (PFGE) showed that no mini-chromosomes are comprised in the genome. Even the smallest chromosome was 5 to 6 Mb in size and other larger ones were difficult to clearly resolve by PFGE. Cytological observations by bright-field and fluorescence microscopy on mitotic metaphase revealed that chromosome number is n=10. Interestingly, most chromosomes contained a very large, highly A-T-rich segment that were persistent throughout cell cycle and hence thought to be constitutive heterochromatin. The other chromosomal parts seemed to be G-C-rich. The nucleolar organizer region was easily discerned on a large chromosome based on its characteristic stainability to fluorescent dyes. By measuring axial length of chromosomes, the total genome size was roughly estimated to be 80 to 100 Mb, among the largest in Ascomycota. We hypothesize that *C. orbiculare* has accumulated a large amount of repetitive sequences to constitute heterochromatin, which contributes to the generation of this exceptionally large genome.

22. Changes in gene expression of *Botrytis cinerea* at low temperature and functional analysis of a bacteriorodopsin-like gene. S. Ish - Shalom, and A. Lichter* Department of Postharvest Science, ARO, The Volcani Center, POB 6, Bet Dagan, 50250, Israel [*e-mail: vtlicht@agri.gov.il].

The fungus *Botrytis cinerea* causes the "Gray mold" disease in a large number of plant species. *B. cinerea* is also a major postharvest pathogen, due to its exceptional ability to develop at low temperature, which is the major postharvest tool used to maintain the quality of fresh produce. The aim of this research is to understand genetic factors which allow *B. cinerea* to develop under low temperature and the working hypothesis is that up regulated genes at low temperature may be involved in mechanisms of cold tolerance. Three methodologies were used to discover cold induced genes: a) genes which are known to be involved in cold tolerance in other organisms; b) genes which originated from cDNA subtraction; c) microarray data using a Nimblegen platform. Gene expression during growth was compared at 4ºC and 22ºC after 1, 4, 10 and 24 h. The microarray data showed that many genes which changed significantly ($P<0.01$), do not have assigned function and among genes with assigned functions, many are not known to be involved in cold response. Diverse cellular functions are influenced but stress response is under-represented. One of the genes which was markedly unregulated at low temperature was a bacterio-Rhodopsin-like (bR) gene that serves as light proton pumps in bacteria was chosen for functional analysis. Knockout strains of bR show attenuated growth at low temperature and were extremely sensitive to osmotic stress.

23. Mapping a vegetative incompatibility locus in the plant pathogen *Botryotinia fuckeliana* using high throughput sequencing technologies. Saadiah Arshed[1,2], Erik Rikkerink[2], Murray Cox[3], Michael Pearson[1], James Lee[1,2], Ross Beever[4] and Matthew Templeton[2]. [1]The University of Auckland, Auckland, New Zealand. [2]The New Zealand Institute for Plant & Food Research, Auckland, New Zealand. [3]Massey University, Palmerston North, New Zealand. [4]Landcare Research, Auckland, New Zealand.

*V*egetative *i*ncompatibility (VI) in *Botryotinia fuckeliana* is controlled by at least 7 loci termed *vic* (*v*egetative *ic*ompatibility) or *het* (*het*erokaryon incompatibility). Our main objective is to map a single *vic* locus that regulates VI in near isogenic lines of interacting compatible and incompatible *B. fuckeliana* strains. Progeny generated from multiple backcrosses were pooled into two vegetative compatibility groups, sequenced using Illumina technology and mapped to a reference genome for SNP analyses. A small proportion of unique SNPs was identified that were diagnostic of the two pools and found to be clustered into a few candidate genomic regions. The most promising candidate identified by BLASTp and Pfam is a predicted protein that contains domain architectures implicated with VI in *Podospora anserina* and *Aspergillus* spp., including the NACHT family of NTPases, ankyrin repeats and a putative serine esterase. We are currently verifying the candidate status of this protein, using PCR to survey the progeny for alleles.

24. The fludioxonil induced phosphoproteomes of the phytopathogenic fungi *Alternaria brassicicola* and *Botrytis cinerea*. Marlène Davanture[1], Benoît Valot[1], Claire Campion[2], Jérôme Dumur[2], Nelly Bataillé-Simoneau[2], Michel Zivy[1], Philippe Simoneau[2], and S. Fillinger[3] [1]PAPPSO, INRA-CNRS-University Paris XI-Agro ParisTech, Gif-sur-Yvette, France; [2]UMR PaVé, INRA-University Angers, France; [3]BIOGER CPP, INRA Versailles-Grignon, France

Protein phosphorylation and dephosphorylation are cellular processes rapidly induced by external *stimuli* adapting regulatory circuits and enzymatic functions to changing environmental conditions. Signal transduction (ST) pathways necessary for signal perception and phosphorylation cascades are involved in many physiological processes such as development, stress adaptation, virulence etc. ST components may either constitute targets for agronomic fungicides or mediate resistance to these compounds. In order to identify and to compare the proteins involved in transducing the signal perceived after phenylpyrrol treatment in two phytopathogenic fungi, we established a gel-free phosphoproteomic approach for systematic identification of phosphorylated peptides in *Alternaria brassicicola* and *Botrytis cinerea*, treated or not with fludioxonil. The gel-free phosphoproteomic approach combines two sequential steps after trypsin digestion : i) SCX chromatography (strong cation exchange); ii) IMAC (Immobilized metal affinity chromatography) for the enrichment of phosphopeptides prior to LC-MS/MS analysis. Preliminary experiments revealed that among the high number of identified phosphoproteins (500-600 and >800 for *Ab* and *Bc* samples, respectively), more than 12% were found specifically phosphorylated and 8% dephosphorylated following the fludioxonil treatment. Among the functional categories identified, we noticed a high proportion of proteins with regulatory functions (transcription, translation etc.) or involved in signal transduction. Particular cellular functions affected by (de)phosphorylation under these conditions concern the cell envelope and transport across it. Higher proportions of phosphorylated proteins compared to dephosphorylated proteins following fludioxonil treatment were found for the functional categories of metabolism and energy production, especially lipid metabolism, as well as of cytoskeleton and cell cycle. We are currently analyzing additional samples to confirm this analysis.

25. Re-sequencing of Verticillium dahliae isolates reveals high genome plasticity. Ronnie de Jonge and Bart Thomma Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands

Verticillium spp. are soil-borne plant pathogens that are responsible for Verticillium wilt diseases on a wide range of host plants. They belong to the class of Deuteromycetes, a class of fungi for which no known sexual stage has been found. In our research we focus on V. dahliae, a plant pathogenic species within the Verticillium genus. In order to perform population genetics and comparative genomics, we have used massive parallel sequencing to determine the genome sequences of 11 V. dahliae isolates in addition to the publicly available genome of the V. dahliae isolate VdLs17. The isolates were selected based on whether they are pathogenic or not on Arabidopsis and tomato, and on aggressiveness. Sequences were assembled using a combination of mapping tools (SOAPaligner, Maq) and de novo assemblers (SOAPdenovo and Velvet). In our initial genome comparisons, whole genome mapping was performed to investigate structural differences on the scaffold level, and nucleotide diversity on the gene level, between the newly sequenced isolates and VdLs17. For most isolates, the largest percentage of reads (70-80%) could be mapped with high confidence and similarity to VdLs17. Furthermore, de novo assembly of the non-mappable reads and subsequent comparisons between isolates demonstrated high sequence diversification of some regions, as well as complete loss of other regions, demonstrating a high degree of plasticity of the V. dahliae genome.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

26. Genome reconstruction of oomycete pathogens. Michael F Seidl[1], and Berend Snel. Theoretical Biology and Bioinformatics, Utrecht University & Centre for BioSystems Genomics (CBSG), Wageningen, The Netherlands. [1]m.f.seidl@uu.nl

Oomycetes are the causal agents of devastating diseases on plants, animals and insects. Recently several genomes of these pathogens became available. These fungi-like organisms have large and flexible genomes with expanded gene families that are implied to play an important role in the host-pathogen arms race. Hence, we want to systematically investigate when gene families in these pathogens duplicated, e.g. in the common ancestor or continuously along the tree, and conversely, whether the adaption of oomycetes to their host and life style is in part due to the loss of certain families. Therefore, we analyzed the predicted proteomes of six pathogenic oomycetes and four non-pathogenic sister taxa (diatoms, brown and golden-brown algae). We constructed ~12,000 multi-species gene (protein) families as well as their gene (protein) trees and reconciled these with a reliable species phylogeny. The inferred evolutionary events were projected onto the species tree. We observed a high number of duplications and losses, especially within the oomycetes, that shape the genome content of the extant organisms. The evolutionary signature of duplications and losses along the branches of the species tree differed significantly between functional classes. Our results corroborate and generalize recent observation in the study of individual gene families. Moreover, this will aid in the understanding of the evolutionary processes that shape the genome content of extant oomycetes.

27. Antimicrobial activity and polyketide synthase gene cloning of the stone fruit pathogen *Monilinia fructicola.* Fang-Yi Yu[1], Chien-Ming Chou[1], Pei-Ling Yu[1], Richard M. Bostock[2] and Miin-Huey Lee[1] [1]Department of Plant Pathology, National Chung-Hsing University, 250 Kuao-Kuang Rd., Taichung 402, Taiwan. [2]Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616

Monilinia fructicola causes blossom blight and fruit rot of stone fruits. Infected fruit are colonized by this fungal pathogen, which eventually forms black stroma as resting structures. A slowly growing isolate, TW5-4, from our *M. fructicola* collection formed dark colonies and expressed strong antimicrobial activity against phytopathogenic fungi and bacteria. In addition, an albino mutant, TW5-4MW, was spontaneously generated from TW5-4, and displayed normal growth but had reduced antimicrobial activity. We have partially purified antimicrobial compounds from cultures and have preliminary evidence that they are aromatic. An early study (Sassa et al. 1983) identified an antimicrobial phenolic octaketide (monilidiol) from benomyl-resistant isolates of *M. fructicola*. Biosynthesis of fungal melanins is known to require the involvement of polyketide synthases (PKS) in the primary biosynthesis step. To elucidate the relationship of stroma formation, pigmentation and antimicrobial activity, twelve partial PKS genes of *M. fructicola* were isolated by degenerate PCR and their expression analyzed by semi-quantitative rt-PCR. Results indicate that some of the 12 PKS genes are differentially expressed in the three isolates examined: TW5-4, TW5-4MW and M1 (a representative wild-type strain of *M. fructicola*). Functional analyses of the PKS genes in fungal development, pathogenicity and antimicrobial activity using gene silencing are underway.

28. Transcriptome profiles of *Fusarium graminearum* during infection of wheat, barley and maize. Linda Harris, Margaret Balcerzak, Danielle Schneiderman, Anne Johnson, and Thérèse Ouellet. Eastern Cereal & Oilseed Research Centre, Agriculture & Agri-Food Canada, Ottawa, ON, K1A 0C6, Canada, Linda.Harris@agr.gc.ca .

The interaction between host and pathogen can be dissected using transcriptome studies and can lead to the development of new strategies for pathogen control. We compared gene expression profiles of *Fusarium graminearum* growing on three susceptible monocots to identify genes required for early infection of diverse hosts. RNA profiling has been performed using a custom 4x44K Agilent microarray platform (three oligomers representing each of 13,918 predicted *F. graminearum* ORFs), detecting gene expression at 1, 2 and 4 days after inoculation of wheat and barley heads and maize ears. Surveying three biological replicates of each time point collection and eliminating plant cross-hybridizing probes, the expression of 6879, 4602, and 2389 *F. graminearum* genes was documented in inoculated wheat, barley, and maize, respectively. A majority of the expressed fungal genes are observed in all three hosts. However, a small subset of genes shows a host-preferential expression pattern. Quantitative PCR analyses have validated the microarray gene expression profiles of selected genes.

29. Correlating fungal growth and biotope to genome content using the FUNG-GROWTH and CAZy databases. Ronald P. de Vries(1), A. Wiebenga(1), Vincent Robert(1), Pedro M. Coutinho(2), Bernard Henrissat(2) (1)CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; (2)AFMB, Marseille, France; e-mail: r.devries@cbs.knaw.nl

Fungal genome sequences demonstrate the potential to utilize a variety of different carbon sources. Natural carbon sources for many fungi are based on plant biomass and often consist of polymeric compounds, such as polysaccharides. They cannot be taken up by the fungal cell and are extracellularly degraded by a complex mixture of enzymes. Plant polysaccharide degrading enzymes have been studied for decades due to their applications in food and feed, paper and pulp, beverages, detergents, textile and biofuels. These enzymes have been classified based on amino acid sequence modules (www.cazy.org). Based on the hypothesis that fungal genomes have evolved to suit their ecological niche, we have performed a comparative study using >90 fungal species. In this study we have compared growth profiles on 35 different carbon sources (consisting of mono-, oligo- and polysaccharides, lignin, protein and crude plant biomass) to the CAZy annotation of the genomes and the natural biotope of the species to identify correlations between growth and genomic potential. In addition we have compared growth on monosaccharides to the presence/absence of related metabolic pathways. Highlights of these comparisons as well as the FUNG-GROWTH database (www.fung-growth.org) will be presented.

30. Pathogens of land and water: functional genomics of plant and animal pathogenic oomycetes. Rays H.Y. Jiang[1], Brian J. Haas[1], Sean Sykes[1], Broad Genome Annotation Group[1], Sarah Young[1] , Irene De Bruijn[2], Pieter van West[2], Chad Nusbaum[1], Brett Tyler[3] and Carsten Russ[1] [1] Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA [2] University of Aberdeen, Aberdeen AB25 2ZD, UK [3] Virginia Bioinformatics Institute, Blacksburg, VA 24061, USA

Saprolegnia parasitica is an oomycete pathogen that causes severe diseases in fish, amphibians and crustaceans, resulting in major annual losses to aquaculture and damage to aquatic ecosystems. It represents the first animal oomycete pathogen genome to be sequenced. We annotated the 53 Mb genome using *ab initio* methods and RNA- seq data from multiple life stages. We report on the genomic landscape and analysis comparing *Saprolegnia* with plant-pathogenic *Phytophthora* species. The canonical host-targeting domains found in plant pathogen oomycetes appear to be absent in *Saprolegnia*. Nevertheless, we found several effector candidates with variant-RXLR motifs, and showed experimentally that they target animal host cells. The largest plant pathogen effector families in *Phytophthora*, such as RXLR, crinkler and Necrosis Inducing Proteins (NIP) are absent in *Saprolegnia*. In contrast, *Saprolegnia* possesses one of largest sets of protease genes among eukaryotes, with about 300 more than typical fungal species. RNA-seq data show a wave-like deployment of proteases at different points during infection. *Saprolegnia* also has a massive kinome of 619 genes, similar in size to mammalian kinomes, 20% of which are induced upon infection. Surprisingly, *Saprolegnia* has ~50 protein domains that otherwise only occur in animals, e.g. disintegrins and Notch-like proteins, which may modulate host cells by molecular mimicry. Comparison of Saprolegnia and Phytophthora indicates that different host cellular environments have greatly shaped the evolution of plant and animal pathogen genomes.

31. Systematic deletion analysis of *Aspergillus nidulans* kinase genes. [1]Colin P. De Souza, [1]Shahr B. Hashmi, [1]Aysha H. Osmani, [2]Carol S. Ringelberg, [2]Jay C. Dunlap and [1]Stephen A. Osmani. [1]The Ohio State University, Columbus, OH, USA. [2]Dartmouth Medical School, Hanover, NH, USA. (osmani.2@osu.edu)

Phosphorylation mediated through kinase enzymes is important to the regulation of virtually all eukaryotic processes. In the filamentous fungi analysis of these important regulatory enzymes has been limited to specific kinases identified through biochemical or genetic approaches or by sequence similarities to kinases in other organisms. However, recent advances in gene targeting and the availability of pre-made deletion constructs makes possible the global analysis of all kinases in Aspergillus nidulans. We report here the deletion and primary characterization of all protein kinase, histidine kinase and PI3/PI4 kinase encoding genes, totaling 130 deletions, in this model fungus. Each gene has been deleted and defined as either essential or not essential. Non-essential haploid deleted strains have been tested for conditional phenotypes in response to numerous cellular stress conditions. In addition, for all 24 of the essential kinase genes, the terminal growth and cell cycle phenotype has been defined using heterokaryon rescue and microscopic analysis of DAPI stained cells. This global analysis has confirmed the phenotypes of previously studied kinase genes and has expanded the number of kinase genes that have now been characterized in A. nidulans by 82. The deleted strains have been deposited at the FGSC and provide a powerful resource for analysis of processes regulated by phosphorylation in fungi. (Funded by NIH Program Project grant GM068087)

32. A method for accurate prediction of the size of secondary metabolite clusters in *Aspergillus nidulans.* Mikael R. Andersen[1], Jakob B. Nielsen[1], Mia Zachariassen[1], Tilde J. Hansen[1], Kristian F. Nielsen[1], and Uffe H. Mortensen[1]. [1]Center for Microbial Biotechnology, Technical University of Denmark, Denmark.

Fungal secondary metabolites (SMs) are receiving increasing interest due to their role as bioactives, ranging from antibiotics over cholesterol-lowering drugs to food toxins. The identification of SMs and their biosynthetic gene clusters are thus a major topic of interest. Identifying these genes is a tedious and time-consuming affair, with the standard method requiring the knockout of genes on both sides of putative SM synthases. Furthermore, one does not know the number of genes in the cluster and thereby extent of this work before starting the experiment. In this work, we present an algorithm for prediction of the size of SM clusters in *Aspergillus nidulans*. The method is based on an gene expression catalog of >60 transcriptome experiments, using a diverse set of strains, media, carbon sources, and solid/liquid cultivations. Furthermore, the method is independent of the quality of annotation. Application of the algorithm has allowed the accurate prediction of the number of included genes in well-characterized gene clusters. including the 25 genes of the sterigmatocystin cluster and the emericellamide cluster (4 genes). The method has provided strong predictions of unknown clusters, some of which we have verified experimentally and identified the corresponding metabolites.

33. Genome analysis of a strain from the UK blue 13 clonal lineage of *Phytophthora infestans* reveals significant genetic and expression polymorphisms in effector genes. Liliana M. Cano[1], Sylvain Raffaele[1], Ricardo Oliva[1], David Cooke[2], Paul Birch [2]and Sophien Kamoun [1] [1] The Sainsbury Laboratory, JIC Norwich Research Park. NR47UH, Norwich, UK. [2] SCRI, Invergowrie, Dundee. DD25DA, Scotland, UK.

Phytophthora infestans is an oomycete pathogen that causes the devastating late blight disease in potatoes. In 2005, a clonal lineage of the A2 mating type, termed genotype blue 13, was identified in the UK and now this strain has become the most prevalent in the country. *P. infestans* blue 13 strains are characterized by an increased aggressiveness and virulence on several resistant potato varieties. Genome analysis of *P. infestans* blue 13 UK3928 strain revealed regions containing RXLRs with copy number variation (CNV) represented by increased depth of coverage. In addition, a whole-genome microarray screen allowed the detection of specifically induced genes on potato with no induction in the less virulent *P. infestans* reference strain T30-4. Our findings suggest that *P. infestans* blue 13 exhibit significant CNV and expression polymorphisms in effector genes. A better understanding of the genetic variation of *P. infestans* blue 13 will help to provide clues of the evolution of virulence of this epidemic disease.

34. Whole-genome duplication in Mucoromycotina? A.Salamov[1], A.Kuo[1], S.Torres-Martinez[2], L.Corrochano[3], I.Grigoriev[1] [1] DOE Joint Genome Institute, Walnut Creek, USA [2] Universidad de Murcia, Spain [3] Universidad de Sevilla, Spain

Mucoromycotina is one of three clades of former phylum of Zygomycota. The genome of one sequenced Mycoromycotina species - Rhizopus oryzae - was shown to have the features of whole-genome duplication (WGD) (Ma et al, 2009). JGI have sequenced two other species from the same clade - Phycomyces blakesleeanus and Mucor circinelloides. From the comparative analysis of these three genomes we found several evidences including higher number of loosely conserved duplicated regions, which indicate that in addition to lineage-specific WGD in R.oryzae, the more ancient WGD event may occurred at the root of Mycoromycotina.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

35. *Fusarium* Mitochondrial Genome Dynamics: Horizontal Transmission, Gene Duplication and Degeneration. Rasha M. Alreedy and John C. Kennell. Saint Louis University, St. Louis, MO

With few exceptions, mitochondrial (mt) genomes of fungi, plants, and animals encode the same set of ~13 genes, each of which play a direct role in oxidative respiration or ATP synthesis. The annotation of mtDNAs of *Fusarium* species revealed two novel open reading frames (ORFs) that are not found in other fungal mtDNAs. The first ORF (designated ORF151) is highly conserved among *Fusarium* species and derives from the partial duplication of the last exon of *cox1*. Other small ORFs that derive from the duplication of 3' end of highly conserved mt genes are also detected, suggesting that they may have been generated by a common mechanism. The second ORF (designated BigORF) is predicted to encode an exceptionally large, unidentified polypeptide (>2000 a.a.). The region encoding the BigORF is located between *rnl* and *nad2* and appears to have been introduced via horizontal transmission prior to *Fusaria* speciation. The region has a sporadic distribution within all species that have been examined, with some strains experiencing a complete loss and others showing varying degrees of degeneration. In strains that retain the BigORF, there is a surprisingly low level of conservation suggesting that the region could serve as a strain-specific molecular marker. The unexpected finding of two conserved ORFs in *Fusarium* species is in contradiction to the wide-scale reduction of mt genome size and gene content experienced by mtDNA lineages and future studies could reveal mechanisms associated with both the gain and loss of mitochondrial DNA sequences.

36. The genome sequence of *Leptosphaeria biglobosa* 'canadensis', a pathogen of oilseed Brassicas. Angela P. Van de Wouw[1], Marie-Helene Balesdent[2], Jonathan Grandaubert[2], Thierry Rouxel[2] and Barbara J. Howlett[1] [1] School of Botany, the University of Melbourne, Parkville, VIC, 3010 Australia [2] INRA-Bioger, Avenue Lucien Brétignières, BP 01, 78850 Thiverval-Grignon, France

Leptosphaeria maculans and *L. biglobosa* comprise a species complex associated with disease of crucifers including *Brassica napus* (canola, oilseed rape) and *B. juncea* (Indian mustard). The genome of *L. maculans* is compartmentalized into GC-rich and AT-rich blocks (Rouxel et al 2010 manuscript accepted). The AT-rich blocks are gene–poor and riddled with degenerated transposable elements and host putative effector genes that encode small secreted proteins (SSPs). We have sequenced the genome of *L. biglobosa* 'canadensis' using paired 75 bp Illumina reads. The genome was assembled into 2287 contigs with a median length of 5460 bp, a maximum length of 136,419 bp and an overall size of 28.7 Mb. The genome does not appear to be compartmentalized into AT-rich and GC- rich blocks, nor contain retrotransposons. A third of 52 putative SSPs located in AT-rich blocks of *L. maculans* have a homologue (>65% sequence identity) in *L. biglobosa*. A further 28% of the SSPs from AT-rich blocks of *L. maculans* have potential homologues (35-65% sequence identity). Of the 529 SSPs in GC-rich blocks of *L. maculans*, 60% have a definite homologue with a further 23% having potential homologues in *L. biglobosa*. Lastly, the gene order in both species seems generally conserved.

37. Control and distribution of chromatin domains in *Neurospora crassa*. Zachary A. Lewis, Keyur K. Adhvaryu, Shinji Honda, Kirsty S.F. Jamieson, Michael R. Rountree, Andrew D. Klocko and Eric U. Selker. Institute of Molecular Biology, University of Oregon, Eugene, OR 97403. zlewis@uoregon.edu

Eukaryotic genomes are composed of distinct structural and functional domains marked by various covalent modifications of histone proteins and, in some organisms, by methylation of cytosine bases in DNA. Gene-rich euchromatin is thought to exist in a relatively open conformation, facilitating DNA transactions such as transcription, whereas the gene-poor heterochromatin is more condensed and is a poor substrate for DNA-based transactions. We performed genomic analyses to determine the distribution and molecular composition of heterochromatin domains in Neurospora. We show that heterochromatin domains are directed by A:T-rich DNA (i.e. products of the genome defense system repeat-induced point mutation) and are composed of histone H3 methylated on Lysine-9 (H3K9me3), Heterochromatin Protein-1 (HP1), and DNA methylation. These and other studies revealed that A:T-rich DNA recruits the histone methyltransferase DIM-5, which catalyzes tri-methylation of H3K9. H3K9me3 then directs DNA methylation by recruiting a complex containing HP1 and the DIM-2 DNA methyltransferase. To identify additional genes that regulate DNA methylation, we performed a genetic selection for mutants defective in DNA methylation (*dim*). This selection uncovered a group of genes that encode components of a DIM-5-containing complex (named DCDC) and are essential for H3K9 methylation and DNA methylation. We show that one component of the complex, DIM-7, directs DIM-5 to appropriate regions of the genome. We will present our ongoing analyses of the distributions, functions, and control of chromatin domains in Neurospora.

38. Comparative analysis of mitochondrial genomes from *Aspergillus* and *Penicillium* spp. Vinita Joardar, Suman Pakala, Jessica Hostetler, Suchitra Pakala, Natalie Fedorova and William Nierman J. Craig Venter Institute, Rockville MD, USA.

Fungal mitochondrial genes are widely used in population and phylogenetic studies and have been linked to virulence and senescence in some fungi. While multiple nuclear genomes are available for *Aspergillus* spp., few annotated mitochondrial genomes have been published for these organisms. We report here the complete sequence of 6 mitochondrial genomes of *Aspergillus* as well as 3 *Penicillium* spp.obtained by Sanger sequencing. While core gene content and synteny are well conserved within each genus, the genomes sizes range considerably from 24,658 to 35,056 bp in *Aspergillus* spp. and from 27,017 to 36,351 bp in *Penicillium* spp. The core mitochondrial genome includes the 14 genes involved in oxidative phosphorylation, a complete set of tRNAs, the small and large subunits of ribosomal RNA and the ribosomal protein S5, all encoded on the same strand. The differences in size correlate with the number of introns and the number of accessory genes present in the genome. The smallest genomes do not contain introns in the protein-coding genes whereas the larger genomes contain as many as 9 introns. Accessory genes include intron-encoded endonucleases, DNA and RNA polymerases and hypothetical proteins. The comparative and phylogenetic analysis of these and related publicly available mitochondrial genomes are presented. Funding: NIAID

39. The genome of the Dothideomycete forest pathogen *Dothistroma septosporum*. Rosie E. Bradshaw[1], Andrea Aerts[2], Hui Sun[2], Murray P. Cox[1], Pranav Chettri[1], Stephen B. Goodwin[3] and Igor V. Grigoriev[2]. [1] Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand. [2] DOE Joint Genome Institute, Walnut Creek, CA 94598, USA. [3] USDA-ARS/Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907- 2054, USA.

Dothistroma septosporum causes Dothistroma needle blight of pines and has a worldwide distribution, but has recently reached epidemic status in parts of Europe and Canada. As part of an initiative by the Dothideomycetes Comparative Genomics Consortium, the genome of *D. septosporum* was sequenced by the DOE Joint Genome Institute (JGI) to 34x coverage. The 30.2-Mb genome assembly consists of 20 scaffolds, 13 of which are 1 Mb or larger with telomere sequence at one or both ends. Sizes of the 13 largest scaffolds are similar to those of chromosomes predicted from PFGE analysis. A total of 12580 genes was predicted using the JGI annotation pipeline and are available via the JGI MycoCosm portal at http://www.jgi.doe.gov/Dothistroma. Availability of this genome will facilitate discovery and analysis of potential effector genes involved in communication between the pathogen and its host. It will allow us to explore the genetics of secondary metabolite production in *D. septosporum*, in particular of dothistromin, a toxin produced in diseased pine needles. Comparative genomics analyses with other sequenced Dothideomycetes will help to determine distinctive features of a forest foliar pathogen.

40. Comparative genomics of Dothideomycetes plant pathogens. Robin Ohm[1], Andrea Aerts[1], Asaf Salamov[1], Stephen B. Goodwin[2], Igor Grigoriev[1] raohm@lbl.gov [1] Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598, USA [2] USDA-ARS, Crop Production and Pest Control Research Unit, 915 West State Street, Purdue University campus, West Lafayette, IN 47907-2054, USA

The Dothideomycetes are one of the largest and most diverse groups of fungi. Many are plant pathogens and pose a serious threat to agricultural crops, whether for biofuel, food or feed. Eight genomes of fungi belonging to this group have been sequenced, allowing comparative genome analysis: *Mycosphaerella graminicola*, *Stagonospora nodorum*, *Pyrenophora tritici-repentis* (all pathogens of wheat), *Alternaria brassicicola* (pathogen of Brassica species), *Cochliobolus heterostrophus* (pathogen of corn), *Mycosphaerella fijiensis* (pathogen of bananas), *Dothistroma septosporum* (pathogen of pine trees) and *Septoria musiva* (pathogen of poplar trees). Chromosome content of these organisms is highly conserved; rearrangements have taken place mostly within, rather than between, chromosomes. In *M. graminicola*, several chromosomes have previously been identified as dispensable and on a genomic level show a lower GC content and gene density, higher repeat content and an under representation of proteins involved in metabolism and transcriptional regulation. These chromosomes may have originated by transfer from an unknown donor; a similar effect is observed for scaffolds of other Dothideomycetes. Compared to other fungi, Dothideomycetes have significantly more proteins with: a histidine kinase signaling domain; an ATP binding region; and a peptidase C19 domain. On the other hand, proteins with an ABC transporter domain and those with a GTP binding region are under represented in the Dothideomycetes. Several clusters of orthologous proteins are specific to the Dothideomycetes and have no previously described conserved domain. These results offer valuable insights into fungi of the class Dothideomycetes and their method of pathogenicity.

41. Genome sequencing classical mutants in Neurospora crassa: The burden of proof. Kevin McCluskey, Aric Wiest, Joel Martin, Wendy Shackwitz, Igor Grigoriev, Scott Baker. University of Missouri- Kansas City, Fungal Genetics Stock Center. US DOE JGI, Walnut Creek

The genomes of multiple classical mutants of *Neurospora crassa* were sequenced at the JGI revealing a high amount of genome variability among these strains. Both SNP and indel distribution were analyzed with reference to genetic mapping data to identify mutations against a background of genome variability. The ability to compare among multiple strains leveraged the analysis and allowed identification of putative mutations in multiple strains. The identification of hundreds of thousands of SNPs and indels allowed improvements to gene annotation and provided insight into the co-inheritance of large regions of the genome. The inclusion of a *N. crassa* strain carrying the Sk-2 region introgressed from *N. intermedia* allowed unambiguous delineation of the Sk-2 region and provides insight into the genes potentially responsible for the meiotic drive function. The impact of back-crossing into the reference genome is elucidated by the sequence of this and other strains. Several mutations putatively identified have other alleles that have been characterized at the molecular level. The similarity of phenotype between classical and knock-out mutants is strongly suggestive that these mutations are accurately identified by whole genome sequencing. The presence of significant numbers of mutations in these strains suggests that classical mutant strains carry a burden of neutral or unselected mutations. Additional insight into selection mechanisms for neutral mutations and their impact on protein structure will be presented.

42. Phylogeny and comparative genome analysis of Basidiomycete fungi. Robert Riley, Asaf Salamov, David Hibbett, Igor Grigoriev DOE Joint Genome Institute

Fungi of the phylum Basidiomycota, make up some 37% of the described fungi, and are important from the perspectives of forestry, agriculture, medicine, and bioenergy. This diverse phylum includes the mushrooms, wood rots, plant pathogenic rusts and smuts, and some human pathogens. To better understand these important fungi, we have undertaken a comparative genomic analysis of the Basidiomycetes with available sequenced genomes. We report a detailed phylogeny that resolves previously unclear evolutionary relationships. We also define a 'core proteome' based on protein families conserved in all organisms. We identify key expansions and contractions in protein families that may be responsible for the degradation of plant biomass such as cellulose, hemicellulose, and lignin. Finally, we speculate as to the genomic changes that drove such expansions and contractions.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

43. Genome sequence and genetic linkage analysis of Shiitake mushroom *Lentinula edodes.* H.S. Kwan, C.H. Au, M.C. Wong, J. Qin, I.S.W. Kwok, W.W.Y. Chum, P.Y. Yip, K.S. Wong, L. Li, Q.L. Huang, W.Y. Nong The Chinese University of Hong Kong, Hong Kong SAR, PRC

Lentinula edodes (Shiitake/Xianggu) is an important cultivated mushroom. Understanding the genomics and functional genomics of *L. edodes* allows us to improve its cultivation and quality. Genome sequence is a key to develop molecular genetic markers for breeding and genetic manipulation. We sequenced the genome of *L. edodes* monokaryon L54A using Roche 454 and ABI SOLiD genome sequencing. Sequencing reads of about 1400Mbp were *de novo* assembled into a 39.8Mb genome sequence. We compiled the genome sequence into a searchable database with which we have been annotating the genes and analyzing the metabolic pathways. In addition, we have been using many molecular techniques to analyze genes differentially expressed during development. Gene ortholog groups of *L. edodes* genome sequence compared across genomes of several fungi including mushrooms identified gene families unique to mushroom-forming fungi. We used a mapping population of haploid basidiospores of dikaryon L54 for genetic linkage analysis. High-quality variations such as single nucleotide polymorphisms, insertions, and deletions of the mapping population formed a high-density genetic linkage map. We compared the linkage map to the *L. edodes* L54A genome sequence and located selected quantitative trait loci. The Shiitake community will benefit from these resources for genetic studies and breeding.

44. The genetic structure of *A* mating-type locus of *Lentinula edodes.* M.C. Wong[1], C.H. Au[1], D. Bao[2], M. Zhang[2], C. Song[2], W. Song[2], H.S. Kwan[1] [1]The Chinese University of Hong Kong, HKSAR, China. [2]Institute of Edible Fungi, Shanghai, China.

Shiitake mushroom *Lentinula edodes* is a tetrapolar basidimycete that contains two unlinked mating type loci, commonly called the *A* and *B* loci. *A* mating-type genes coded for two different homeodomain transcription factors, termed HD1 and HD2. Here we identified a pair of divergently transcribed homeodomain transcription factor gene (*HD1* and *HD2*) from the first genome sequence of *L. edodes*. The *HD1* and *HD2* sequences cosegragate with mating types revealed by mating analysis. The genomic sequences of the *A* mating-type genes of several monokaryontic strains were obtained by PCR amplification and sequencing. The expression and open reading frames of the genes were confirmed by cloning and analyzing the full length cDNA sequence from the monokaryon L54A. Analysis on the functional domains of the *A* mating-type proteins was also performed. Based on nucleotide sequence comparison with other known tetrapolar and bipolar mushrooms, the *A* mating-type genes is conserved at the homeodomain region. On the other hand, sequence analysis on the flanking region around the *A* mating-type genes revealed unique genomic organization of the locus in *L. edodes*, different from those of other fungi.

45. Comparative genomic and structural investigation of adenylation domains from the nonribosomal peptide synthetases of *Fusarium.* F. T. Hansen[1]; T. V. Lee[2]; T. E. Sondergaard[1]; J. L. Sørensen[1]; H. Giese[1] [1]Department of Animal Health and Bioscience, Aarhus University, Denmark. [2]Structural Biology, SBS, University of Auckland, New Zealand.

Nonribosomal peptide synthetases (NRPS') are multimodular proteins capable of assembling a variety of amino acids into nonribosomal peptides (NRPs) through a thiotemplate system similar to polyketides or fatty acid synthesis. NRPS' are well studied in prokaryotic systems and methods of predicting the amino acid substrate of the adenylation (A) domains of the modules exists, but these are not directly applicable to eukaryotic NRPS' as these are more complex. Little is known of most of *Fusarium* NRPS', their products or what determines the substrate specificity of each module. We present a comparative genetic study of the NRPS' of the publicly available genomes of four *Fusarium* species wherein we found that a number of NRPS genes are conserved throughout some or all of the species. Recently the structure was solved for the first eukaryotic A domain (1) and to expand the understanding of how A domains interacts with amino acid substrates we have initiated a structural study based on x-ray crystallography on selected A domains from *Fusarium graminearum.* (1) T. V. Lee, L. J. Johnson, R. D. Johnson, A. Koulman, G. A. Lane, J. S. Lott & V. L. Arcus. 2010. Structure of a Eukaryotic Nonribosomal Peptide Synthetase Adenylation Domain That Activates a Large Hydroxamate Amino Acid in Siderophore Biosynthesis. JBC. 285, 4,. 2415–2427.

46. Genome sequence and comparative analysis of *Aspergillus oryzae* sake strains. Takanori Nomura [1,2], Tomoaki Fujimura[1,2], Kenta Oda[1,3], Kazuhiro Iwashita[1,2] and Osamu Yamada[2]. [1]Hiroshima university, Hiroshima, Japan. [2] National Research Institute of Brewing, Hiroshima, Japan. [2] SYSMEX CORPORATION, Japan.

Aspergillus oryzae has been used for Japanese traditional fermentation industry and some different strains were selected and used for each product according to their character. In our previous work, we reported the phylogenic analysis of industrial *A. oryzae* strains using DNA microarray and the correlation between the clade and their use. In this work, we performed genome sequence analysis of two *A. oryzae* sake strains belong to two different clade using high-throughput DNA sequencer and compared with the RIB40 genome. We isolated genome DNA from RIB128 and RIBOIS01 strains and applied for 454 genome sequencer. The genes of assembled contigs were further annotated using Spaln and BLAST. As the result, the genome size of RIB128 and RIBOIS01 strains were 36.9 and 37.8 Mbps, and the homology of conserved genes were 99.42 and 99.65 % in average comparing RIB40. Large deletion and insertion, more than 500 bps, was identified in both genomes and 577 (RIB128) and 337 (RIBOIS01) new genes were founded in the inserted regions. Furthermore, several inter chromosomal recombination was identified in both genome. Now we are examining for the second metabolite gene clusters in these genomes.

47. Comparative functional genomics of two *Saccharomyces* yeasts. Maitreya Dunham. University of Washington, Seattle, WA

Although many fungal strains and species have been sequenced, experimental annotation of these genomes has not kept pace. However, functional studies in these genetically diverse isolates could be very informative in understanding their evolution and ecology. We have chosen one of these understudied species, *Saccharomyces bayanus*, in which to investigate these topics. Using a data-driven approach informed by the deep *S. cerevisiae* literature, we collected over 300 gene expression arrays for conditions found to be highly informative in the sister species. Comparison of the gene expression networks between the two species paints a complex picture of conservation and divergence over 20 million years. Further expression analysis in interspecific hybrids has helped determine which of these changes are determined in *cis* and *trans*. We have paired this analysis with additional comparative studies between the species, including ortholog knockout phenotypes and essentiality, DNA replication dynamics, nucleosome profiling, and behavior over experimental evolution timecourses. In all cases, key components show interesting changes, ranging from differences in replication timing of entire chromosome domains to subtle changes in affinity of nutrient transporters. Integration of these datasets with comparative sequence analysis promises to capture a high resolution picture of species-level evolution. We also hope that our methods will be informative for studies in other sequenced but otherwise understudied species.

48. Bulk segregant analysis followed by high-throughput sequencing reveals the Neurospora cell cycle gene, *ndc- 1*, to be allelic with the gene for ornithine decarboxylase, *spe-1*. Kyle R. Pomraning, Kristina M. Smith and Michael Freitag. Program for Molecular and Cellular Biology, Department of Biochemistry and Biophysics, and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

With the advent of high-throughput DNA sequencing it has become straightforward and inexpensive to generate genome-wide SNP maps. Here we combined high-throughput sequencing with bulk segregant analysis to expedite mutation mapping. The general map location of a mutation can be identified by a single backcross to a strain enriched in SNPs compared to a standard wildtype strain. Bulk segregant analysis simultaneously increases the likelihood of determining the precise nature of the mutation. We will present a high-density SNP map between *Neurospora crassa* Mauriceville-1-c (FGSC2225) and OR74A (FGSC987), the strains most typically used by Neurospora researchers to carry out mapping crosses. We demonstrate the utility of our methods by identification of the mutation responsible for the only known temperature-sensitive (ts) cell cycle mutation in Neurospora, nuclear division cycle-1 (*ndc-1*). A single T to C point mutation maps to the gene encoding ornithine decarboxylase (ODC), *spe-1* (NCU01271), and changes a Phe to a Ser residue within a highly conserved motif next to the catalytic residue of the enzyme. By growth on spermidine and complementation with wildtype *spe-1* we showed that the defect in *spe- 1* causes the ts *ndc-1* mutation. We also showed that *ndc-1* is not ts-lethal, as previously reported, but viable after shifting from 37˚ to 22˚. Based on our results we propose to change *ndc-1* to *spe- 1*^{ndc}.

49. Genome-wide expression profiling of transcription factor genes reveals new insights on fugal pathogenicity and stress response in *Magnaporthe oryzae.* Sook-Young Park[1], Se-Eun Lim[1], Jongsun Park[1], Sunghyung Kong[1], Seryun Kim[1], Hee-Sool Rho[1], Yang Kim[2], Kyoung- Young Jeong[1], Jae-Jin Park[1], Junhyun Jeon[1], Myung-Hwan Chi[1], Jaeyoung Choi[1], Soonok Kim[1], Seogchan Kang[3], and Yong-Hwan Lee[1] [1]Dept. of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea; [2]Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea; [3]Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA. sookyp@gmail.com

The availability of genome sequence and expression analyses of predicted genes promise new insights to uncover gene function. In phytopathogenic fungi, although much effort has been focused on understanding the molecular nature of pathogenicity, little is known about transcriptional regulation of pathogenicity-related genes at genome-wide level. Here we describe an expression dynamics of 208 transcription factor genes (TFs) in *Magnaporthe oryzae* under 32 conditions including infection-related developments and abiotic stresses. Data were generated using quantitative real- time PCR method and are publicly accessible online. Expression profiling of TFs allowed regulation-dynamics of the TFs during the given conditions. Functional characterization of two APSES family TFs revealed that expression profiles can be applied to predict the function of TFs. This comprehensive analysis of the TFs would provide a guide map to predict function of TFs and a new paradigm to decipher molecular mechanisms of pathogenicity in *M. oryzae*.

50. Comparative sequence analysis of wheat and barley powdery mildew fungi reveals gene colinearity, dates divergence and indicates host pathogen co-evolution. Simone Oberhaensli, Francis Parlange, Jan P. Buchmann, Fabian H. Jenny, James C. Abbott, Timothy A. Burgis, Pietro D. Spanu, Beat Keller and Thomas Wicker simone.oberhaensli@access.uzh.ch

The two fungal pathogens *Blumeria graminis f.sp. tritici* (*B.g. tritici*) and *hordei* (*B.g. hordei*) cause powdery mildew specifically in wheat or barley. They have the same life cycle, but their growth is restricted to the respective host. We compared the sequences of two loci in both cereal mildews to determine their divergence time and their relationship with the evolution of their hosts. We sequenced a total of 273.3 kb derived from *B.g. tritici* BAC sequences and compared them with the orthologous regions in the *B.g. hordei* genome. Protein coding genes were colinear and well conserved. In contrast, the intergenic regions showed very low conservation mostly due to different integration patterns of transposable elements. To estimate the divergence time of *B.g. tritici* and *B.g. hordei*, we used conserved intergenic sequences including orthologous transposable elements. This revealed that *B.g. tritici* and *B.g. hordei* have diverged about 10 million years ago (MYA), two million years after wheat and barley (12 MYA). These data suggest that *B.g. tritici* and *B.g. hordei* have co-evolved with their hosts during most of their evolutionary history after host divergence, possibly after a short phase of host expansion when the same pathogen could still grow on the two diverged hosts.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

51. Genome expansion and gene loss in powdery mildew fungi reveal functional tradeoffs in extreme parasitism. Pietro Spanu and the BluGen sequencing Consortium. Imperial College London, UK and Others.

Powdery mildews are phytopathogens whose growth and reproduction are entirely dependent on living plant cells. The molecular basis of this lifestyle, obligate biotrophy, remains unknown. We present the genome analysis of barley powdery mildew, *Blumeria graminis* f.sp. *hordei (Blumeria)* , and a comparison with those of two powdery mildews pathogenic on dicotyledonous plants. These genomes display massive retrotransposon proliferation, genome size expansion and gene losses. The missing genes encode enzymes of primary and secondary metabolism, carbohydrate- active enzymes and transporters, probably reflecting their redundancy in an exclusively biotrophic lifestyle. Among the 248 candidate effectors of pathogenesis identified in the *Blumeria* genome very few (<10) define a core set conserved in all three mildews, suggesting that most effectors represent species-specific adaptations.

52. Ancient presence-absence polymorphism of functional whole chromosomes in related pathogen species. Klaas Schotanus 1), Mikkel H. Schierup 2), Bruce A. McDonald 3) and Eva H. Stukenbrock 1) 1) Max Planck Inst. Microbiol., Karl von Frisch Str., D-35043 Marburg, Germany 2) Bioinformatics Res. Center, Aarhus University, CF Moellers Alle 8, DK-8000 Aarhus C, Denmark 3) ETH-Zurich, Inst. Integrative Biol., LFW B16, CH-8092 Zurich, Switzerland Email: klaas.schotanus@mpi-marburg.mpg.de

The wheat pathogen *Mycosphaerella graminicola* contains up to eight dispensable chromosomes. The role of these small chromosomes is unknown. Whole genome sequencing of closely related Mycosphaerella strains from wild grasses revealed that six and four of the small chromosomes are also dispensable in two Mycosphaerella relatives named S1 and S2 infecting wild grasses. The sharing of homologous dispensable sequences suggests that the presence-absence polymorphism of chromosomes is ancient and preserved across species boundaries. PFGE, Southern blots and extensive sequence analyses reveal that the chromosomes not only show polymorphism for their presence, but also frequently undergo structural rearrangements. We ask why the chromosomes are not either fixed or completely lost from the fungal genomes. One hypothesis is that the small chromosomes are easily lost during meiosis however still contain genes which are advantageous under some conditions. We investigate the functional importance of genes on the dispensable chromosomes to understand how natural selection operates on the chromosomes. Our investigations include expression studies and further evolutionary analyses of the genes.

53. A versatile gene expression and characterization system for filamentous fungi. Bjarne Gram Hansen, Morten Thrane Nielsen, Bo Salomonsen, Jakob Blæsbjerg Nielsen, Niels Bjørn Hansen, Kristian Fog Nielsen, Kiran Patil and Uffe Hasbro Mortensen. Technical University of Denmark, Department of Systems Biology, Center for Microbial Biotechnology, Denmark. E-mail: bgha@bio.dtu.dk

Assigning functions to newly discovered genes constitutes one of the major challenges en route to fully exploit the data becoming available from the genome sequencing initiatives. To facilitate the use of filamentous fungi in functional genomics, we present a versatile cloning system that allows gene of interest (GOI) to be expressed from a defined genomic location of *A. nidulans*. By a single USER cloning step, genes are easily inserted into a combined targeting-expression cassette ready for rapid integration and analysis. The system comprises a vector set that allows genes to be expressed from a range of constitutive promoters or from the inducible PalcA promoter. Moreover, by using the vector set, protein variants can easily be made and expressed from the same locus, which is mandatory for proper comparative analyses. Lastly, all individual elements of the vectors can be substituted for other similar elements ensuring the flexibility of the system. We have demonstrated the high-throughput potential of the system by transferring more than 100 genes from filamentous fungi into *A. nidulans*. In addition, we produce defined mutant derivatives of selected GOI which allows an in-depth analysis of the GOI. Importantly, since the vector set is constructed in a flexible manner, it can without problems be modified to allow specific integration of GOI into other fungi. The strategy for gene characterization presented here is therefore widely applicable and should greatly facilitate assignment of gene functions in fungi where the genetic tool-box is poorly developed.

54. New resources for functional analysis of omics data for the genus *Aspergillus.* B.M. Nitsche [1], A.F.J. Ram [1,2], V. Meyer [1,2], J.R. Wortman [3] [1] Institute of Biology, Leiden University Sylviusweg 72, 2333 BE Leiden, the Netherlands, [2] Kluyver Centre for Genomics of Industrial Fermentation, Sylviusweg 72, 2333 BE Leiden, the Netherlands and [3] Department of Medicine Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, USA

Detailed and comprehensive genome annotation can be considered a prerequisite for effective analysis and dissection of omics data. As such, Gene Ontology (GO) annotation has become a well-accepted framework for functional annotation. The genus *Aspergillus* comprises fungal species that are important model organisms, plant and human pathogens as well as industrial workhorses. GO annotation based on computational prediction and manual curation has so far only been available for one of its species, namely *A. nidulans*. Based on protein homology and synteny, we have mapped 97% of the 3,498 *A. nidulans* GO annotated genes to at least one of seven other *Aspergillus* species: *A. niger, A. fumigatus, A. flavus, A. clavatus, A. terreus, A. oryzae* and *Neosartorya fischeri*. Furthermore, we developed the web application FetGOat, which can be used to perform GO enrichment analysis for eight *Aspergillus* species. We have benchmarked the newly mapped GO annotation and the web application FetGOat by analyzing two recently published microarray datasets and comparing the results to those obtained with two freely available analysis tools, Blast2GO and GSEA.

55. Targeted activation of the polyketide synthase 9 gene cluster in *Fusarium graminearum*. Jens Laurids Sørensen[1]; Frederik Teilfeldt Hansen[1]; Teis Esben Sondergaard[1]; Reinhard Wimmer[2];Aida Droce[1], Henriette Giese[1]; Rasmus John Norman Frandsen[3] [1]Department of Animal Health and Bioscience, Aarhus University, Denmark. [2]Department of Life Sciences, Aalborg University, Aalborg, Denmark. [3]Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark.

The important pathogen of maize and wheat, *Fusarium graminearum*, has similar to other filamentous fungi the potential to produce a wide array of secondary metabolites, which is far from fully exploited under laboratory conditions. Genome mining of *F. graminearum* has identified 15 polyketide synthases (PKS), but only four products are known. The remaining genes are either silent under laboratory conditions or are responsible for production of low levels of secondary metabolites. One way for targeted activation of silent pathways is homologues over-expression of putative PKS cluster specific transcriptional regulators. The search for novel secondary metabolites in mutant strains can be hindered by the presence of other metabolites, which may co-elute and thereby camouflage the new compounds. To overcome this obstacle we here describe a method for *F. graminearum*, where the targeted integration of an expression cassette containing the transcription factor for the PKS9 cluster disrupts the function of PKS12, which is responsible for production of the mycelium pigment aurofusarin. This led to a massively enhanced production of three novel compounds, which has not previously been linked to a gene or reported from *F. graminearum*.

56. Comparative phenotyping coupled with high throughput forward genetics and gene deletion strategies reveals novel determinants of pathogenicity in the rice blast fungus *Magnaporthe oryzae*. Janet Wright, Cristian Quispe Jessie Fernandez, David Hartline, Karina Stott, Anya Seng, Jonathan Hinz and Richard A. Wilson. Department of Plant Pathology, University of Nebraska-Lincoln, USA. rwilson10@unl.edu .

To cause rice blast disease, *Magnaporthe oryzae* has distinct morphogenetic stages that allow it to breach the surface of the host leaf and invade the plant tissue. The sugar sensor trehalose-6-phosphate synthase (Tps1) monitors the transition from the nutrient-free surface to the nutrient-rich interior of the leaf and regulates plant infection via a NADP(H)-dependent genetic switch. However, which metabolic and regulatory pathways are required for the fungus to adapt to the fluctuating nutritional environment of the plant host, and how it acquires nutrient during its biotrophic growth phase, is not known. Therefore, using simple plate tests, we sought to determined which biochemical pathways, over- or under-represented in the plant pathogen *M. oryzae* compared to the soil saprophyte *Aspergillus nidulans*, could be required for the rice blast lifestyle. We also compared to wild type the metabolic diversity of key *M. oryzae* regulatory mutants, such as Δtps1 and Δnut1 deletion strains (the latter required for nitrogen source utilization). Finally, we coupled this comparative phenotyping study to high throughput Agrobacterium-mediated forward genetics and gene deletion strategies to rapidly identify and functionally characterize the role of important biochemical and regulatory pathways in disease establishment. In this manner, we report here how carbon catabolite repression and citrate efflux is essential for virulence, and how perturbing histone gene regulation results in severe condial reduction and complete loss of pathogenicity in the devastating rice blast fungus.

57. Is there a light and dark-reaction to environmental cues in fungi? Doris Tisch and Monika Schmoll Vienna University of Technology, Research Area Gene Technology and Applied Biochemistry, Gumpendorfer Strasse 1a, 1060 Wien, AUSTRIA

Perception and proper interpretation of environmental signals is crucial for survival in any natural habitat. In the biotechnological workhorse Trichoderma reesei (anamorph of Hypocrea jecorina) transmission and interpretation of environmental signals via the heterotrimeric G-protein pathway is dependent on the light status. Interestingly, this interconnection of nutrient signaling with light response is to a large extent established by the light regulatory protein ENV1 and the phosducin like protein PhLP1. Lack of either one of the major components of the light response pathway (BLR1, BLR2 or ENV1) or the G-protein beta or gamma subunit and the co-chaperone PhLP1 leads to a partial shut down of a considerable number of genes up-regulated in light and strongly increased light-dependent transcriptional regulation (from 2.8 % to more than 30 % of the genome). These factors are moreover involved in light-dependent regulation of 79 % of all glycoside hydrolase encoding genes, representing all GH-families available in T. reesei. Hence heterotrimeric G-protein signaling exerts its major function in light and signals transmitted via the G-protein pathway are of unequal relevance in light and darkness. We conclude that signal transmission in darkness is performed by different signaling pathways than in light, which enables assignment of a different relevance of a given signal depending on the light status.

58. Gene clusters encoding non-ribosomal peptide sythetases and polyketides synthases in the genome of *Leptosphaeria maculans*. Candace E. Elliott[1], Kim May[1], Dirk Hoffmeister[2], and Barbara J. Howlett[1]. [1] School of Botany, the University of Melbourne, VIC, 3010 Australia [2] Friedrich-Schiller-Universitaet Jena, Department Pharmaceutical Biology at the Hans-Knoell-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany

Known non-ribosomal peptide synthetase (NRPS) genes from other filamentous fungi were blasted against the *L. maculans* genome (Rouxel et al, 2010 manuscript accepted) and homologs were identified. Genomic regions (50 kb) with a NRPS homolog were analysed by FgeneSH and neighbouring genes identified. NRPS and polyketide synthases (PKSs) genes also were identified using domain searches in NCBI and the PKS/NRPS Analysis website (http://www.tigr.org/jravel/nrps/). Thirteen NRPS genes, including *SirP*, involved in sirodesmin biosynthesis, and 12 PKS genes were identified. Ten of the 13 NRPs and eight of the 12 PKSs had matches to PKSs in *Stagonospora nodorum*. Eight of the NRPSs were predicted to reside in a gene cluster based on the presence of neighbouring genes encoding proteins with roles in the biosynthesis of secondary metabolites. Five of the NRPS genes had multiple modules while the remaining eight were monomodular. One monomodular NRPS, named NPS10, had sequence similarity to *Aspergillus nidulans TdiA*, which encodes a bis-indolylquinone synthetase involved in terrequinone A production, and was adjacent to genes involved in secondary metabolism. *L. maculans* isolates with reduced expression of NPS10 have been created and their metabolic profiles are being compared to wild type in order to identify the product of NPS10.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

59. Turning Garbage into Gold: Ectopic Transformants Salvage Strategy for Bidirectional Genetic Study in *Magnaporthe oryzae.* Jaejin Park, Kyoung Su Kim, Junhyun Jeon, Jeil Hong and Yong-Hwan Lee Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea

Being a causal agent of rice blast disease, *Magnaporthe oryzae* is a model plant pathogenic fungus to study fungal pathogenesis. Currently, targeted gene deletion (TGD) is employed as a straightforward way to investigate gene function in reverse genetic study. However, many of filamentous fungi including *M. oryzae* show low frequency of homologous recombination (HR) due to predominance of non-homologous end joining (NHEJ). Although NHEJ-inactivated *M. oryzae* strain had been developed, its use is still hindered by loci-dependency of HR. To complement the TGD strategy and improve the efficiency of genetic study, an add-on system was designed to integrate a forward genetic approach into reverse genetics frame. In our approach, ectopic transformants, by-products of TGD caused by random ectopic integration, are screened for various phenotypes such as reduction in mycelial growth, abnormal pigmentation, abnormal aerial hyphae, reduced conidiation, and abnormal conidiophore morphology. Single ectopic integration was detected via Southern blot analysis and insertion site was identified by inverse-PCR and sequencing. To evaluate the efficacy of this system, a total of 1,151 ectopic transformants were screened and characterized. As a result, 128 transformants (11.1% of total) showed significant phenotypic abnormalities. This combined bidirectional genetic approach would facilitate discovery of novel genes implicated in fungal pathogenesis.

60. MAP Kinase signalling in *Podospora anserina.* Fabienne Malagnac, Hervé Lalucque, Sylvain Brun, Sébastien Kicka and Philippe Silar Université de Paris 7 Denis Diderot, 75205 Paris France Institut de Génétique et Microbiologie, UMR 8621 CNRS Université de Paris 11, 91405 Orsay France

Availability of *Podospora anserina* complete genome sequence, allowed us to identify all the genes encoding MAP kinases (Mitogen Activated Protein). These proteins are involved in signalling pathways through a cascade of three players. At the top of the module, a MAP kinase kinase kinase (MAPKKK) phosphorylates the downstream MAP kinase kinase (MAPKK), which in turn phosphorylates the MAP kinase (MAPK). In the *P. anserina* genome, we found three distinct MAP kinase modules. Knock-outs of the nine genes were performed. All combination of double mutants and the triple MAP kinase deletion mutants were constructed. Phenotypes of the mutants were assayed throughout the lifecycle, towards various stresses and Crippled Growth (CG). The CG alteration corresponds to an epigenetic cell degeneration phenomenon, which results in the inappropriate activation of the MAPK1 MAP Kinase. Our data show that PaMpk2 (the FUS3 orthologue) plays the major role throughout the lifecycle of *P. anserina* , including stress resistance, while the role of PaMpk3 (the HOG1 orthologue) is restricted to hyper-osmotic stress resistance. This systematic investigation of the roles of the three MAP kinases of *P. anserina* led us to test the impact of the two other MAPK signalling modules onto CG triggering and CG propagation.

61. Comparative genomics of the secretome of the Dothideomycete fungus *Cladosporium fulvum*. Ate van der Burgt[1], Harrold van den Burg[1], Ioannis Stergiopoulos[1], Bilal Ökmen[1], Rahim Mehrabi[1], Mansoor Karimi[1], Jerome Collemare[1], Henriek Beenen[1], Gert Kema[2], Rosie Bradshaw[3], Ali Bahkali[4] and Pierre J.G.M. De Wit[1,4*].[1]Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands.[2]Plant Research International, Wageningen, The Netherlands.[3]Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand.[4]King Saud University, College of Science, Botany and Microbiology Department, Riyadh, Saudi Arabia. *Presenting author(pierre.dewit@wur.nl).

The genome size (ca. 70Mb) of the recently sequenced Dothideomycete fungus, *Cladosporium fulvum*, is about twice that of most sequenced fungal genomes including the related fungi *Mycosphaerella graminicola* and *Dothistroma septosporum*, but is about the same as that of *M. fijiensis*. This is mainly due to the high content of sequence repeats (about 40-50%) originating from transposable elements. Bioinformatic analysis revealed that the highest number of effectors homologous to those reported in *C. fulvum* is found in *D. septosporum*. To identify additional effectors and to facilitate the genome annotation, we have performed proteome analyses on the secretome of *C. fulvum* grown *in planta* and *in vitro*. We have identified many novel secreted putative effectors including small cysteine-rich proteins, different classes of proteases, specific cell wall hydolyzing enzymes and proteins of unknown function. For a substantial number of *C. fulvum* secreted proteases intron retention occurs more frequently than in related fungi.

62. Comprehensive Gene Ontology annotation at CGD and AspGD. Marek Skrzypek1, Martha Arnaud1, Jon Binkley1, Gustavo Cerqueira2, Marcus Chibucos2, Maria Costanzo1, Jonathan Crabtree2, Diane Inglis1, Joshua Orvis2, Prachi Shah1, Gail Binkley1, Stuart Miyasato1, Jennifer Russo Wortman2, and Gavin Sherlock1. 1Department of Genetics, Stanford University, Stanford, CA, 2Institute for Genome Sciences, University of Maryland, Baltimore, MD. www.candidagenome.org, www.aspgd.org

Candida Genome Database (CGD) and Aspergillus Genome Database (AspGD) are online resources that collect data on gene products for two important model fungi, Candida albicans and Aspergillus nidulans, respectively. Along with gene names and aliases, general descriptions, and phenotype data, an essential part of the curation process is assigning Gene Ontology (GO) terms, a controlled vocabulary system for annotating gene product molecular functions, biological processes they contribute to, and cellular components where they act. GO annotations are based on published experimental data or computational predictions. CGD/AspGD curators, having reviewed all the gene-specific literature for C. albicans and A. nidulans, have made GO annotations for all the gene products with sufficient supporting information. Experimentally determined GO terms from orthologous genes of S. cerevisiae have also been transferred. The remaining gene products have been annotated to the root terms, molecular function, biological process, or cellular component unknown, to indicate that to the best of our knowledge there is currently no biological data that would allow annotation with a GO term. Curation of new literature is an ongoing process and these annotations are updated as new data emerge. Contact CGD/AspGD curators with suggestions.

63. Chemical rationale for selection of isolates for genome sequencing. Christian Rank*, Thomas Ostenfeld Larsen, Jens Christian Frisvad. Department of Systems Biology, Technical University of Denmark * cr@bio.dtu.dk

The advances in gene sequencing will in the near future enable researchers to affordably acquire the full genomes of handpicked isolates. We here present a method to evaluate the chemical potential of an entire species and select representatives for genome sequencing. The selection criteria for new strains to be sequenced can be manifold, but for studying the functional phenotype, using a metabolome based approach offers a cheap and rapid assessment of critical strains to cover the chemical diversity. We have applied this methodology on the complex *A. flavus/A. oryzae* group. Though these two species are in principal identical, they represent two different phenotypes. This is clearly presented through a correspondence analysis of selected extrolites, in which the subtle chemical differences are visually dispersed. The results points to a handful of strains, which, if sequenced, will likely enhance our knowledge of the chemical potential of *A. flavus/A. oryzae*.

64. The Aspartic Proteinase Family of Three *Phytophthora* Species. Harold J.G. Meijer(1), John Kay(2), Arjen ten Have(3), Francine Govers(1) and Jan A.L. van Kan(1). 1)Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands. 2)School of Biosciences, Cardiff University, Cardiff. U.K. 3)Instituto de Investigaciones Biologicas–CONICET, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina. Email: harold.meijer@wur.nl

Pepsin-like aspartic proteinases (APs) are produced in a wide variety of species and contain conserved motifs and landmark residues. APs fulfil critical roles in infectious organisms and their host cells. *Phytophthora* species are oomycete plant pathogens with major social and economic impact. Several of which have been sequenced. The genomes of *Phytophthora infestans, P. sojae* and *P. ramorum* contain 11-12 genes encoding APs, resolved into 5 clades by phylogenetic analysis. Several subfamilies contain an unconventional architecture, as they either lack a signal peptide or a propart region. One of the *Phytophthora* APs is an unprecedented fusion protein with a putative G- protein coupled receptor as the C-terminal partner. The others appear to be related to well-documented enzymes from other species including a vacuolar enzyme that is encoded in every fungal genome sequenced to date. The oomycetes also have enzymes similar to plasmepsin V, a membrane-bound AP in the malaria parasite *Plasmodium falciparum*, that cleaves effector proteins during their translocation into the host red blood cell. The translocation of *Phytophthora* effectors to host cells is topic of intense research in which APs might be involved.

65. Genome evolution in the Irish potato famine pathogen lineage Sophien Kamoun, The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom.

Eukaryotic plant pathogens, such as oomycetes and fungi, cause highly destructive diseases that negatively impact commercial and subsistence agriculture worldwide. Many plant pathogen species, including those in the lineage of the Irish potato famine organism Phytophthora infestans, evolve by host jumps followed by adaptation and specialization on distinct hosts. However, the extent to which host jumps and host specialization impact genome evolution remains largely unknown. This talk will provide an update on our work on genome evolution in the P. infestans clade 1c lineage. To determine the patterns and selective forces that shape sequence variation in this cluster of closely related plant pathogens, we and our collaborators resequenced several representative genomes of four sister species of P. infestans. This work revealed extremely uneven evolutionary rates across different parts of these pathogen genomes (a two-speed genome). Genes in low density and repeat-rich regions show markedly higher rates of copy number variation, presence/absence polymorphisms, and positive selection. These loci are also highly enriched in genes induced in planta, such as disease effectors, implicating host adaptation in genome evolution. These results demonstrate that highly dynamic genome compartments enriched in non-coding sequences underpin rapid gene evolution following host jumps.

66. Symptom formation of *Sporisorium reilianum* on maize is mediated by secreted effectors. Hassan Ghareeb, Mohammad T. Habib, Yulei Zhao, Jan Schirawski Georg-August-Universität Göttingen, Albrecht-von-Haller Institute, Molecular Biology of Plant-Microbe Interactions, Untere Karspüle 2, 37073 Göttingen, Germany. jschira@uni-goettingen.de

Sporisorium reilianum and *Ustilago maydis* are closely related biotrophic pathogens of maize that cause different symptoms. Upon penetration of seedling plants by *S. reilianum*, fungal hyphae proliferate and spread throughout the plant initially without noticeable impact on plant health. Prominent symptoms are visible at flowering time, when spore-filled sori or leaf-like structures appear in the inflorescences. In addition, infected plants develop more female inflorescences than mock-treated plants. In contrast, an *Ustilago maydis* infection of maize leads to the formation of spore-filled tumors in the vicinity of the site of infection, which can occur on leaves, stems or flowers. To elucidate the molecular basis of the difference in symptom formation, the genome of *S. reilianum* was sequenced and compared to that of *U. maydis* [1]. Both genomes are highly syntenic and most encoded proteins are well conserved. However, a large region on chromosome 19 encoding more than 20 secreted effector proteins shows considerable divergence. We have dissected the contribution of the different fungal effectors of this region to symptom formation of *S. reilianum*. We show that different effectors are responsible for different aspects of the symptoms observed. We have identified one effector whose presence leads to an increase in the number of female inflorescences produced by the plant. However, effector deletion does not affect virulence of the strains. This shows that the different effectors located in the divergence region have a distinct contribution to symptom development of *S. reilianum*. [1] Schirawski et al., 2010. Science 330: 1546-1548.

67. Electrophoretic karyotypes and nuclear DNA contents of *Verticillium albo-atrum* field isolates. Tina Svetek [1], Sebastjan Radisek [2], Borut Bohanec [1], Branka Javornik [1] [1]University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia [2]Slovenian Institute for Hop Research and Brewing, Zalec, Slovenia tina.svetek@bf.uni-lj.si

Our research group is studying the phytopathogenic fungus *Verticillium albo-atrum*, which causes lethal damage to hop (*Humulus lupulus* L.) and thus threatens hop production. Molecular analysis and pathogenicity tests on *V. albo-atrum* have revealed two types of isolates in our hop gardens: isolates (M), which induce mild form of hop wilt, and isolates (L), which kill the plants. Comparison of these two pathotypes with hop *V. albo-atrum* isolates from other European hop growing regions and isolates of *V. albo-atrum* from other hosts, have shown genetic differentiation between all lethal and mild isolates, as well as isolates from other hosts, and a close genetic relationship of our L isolates with L hop isolates from other geographic origins. Furthermore, comparative analysis of mycelium proteomes of the two forms shows similarities among L isolates and among M isolates. Preliminary studies of the genome size of L and M isolates suggest that lethal isolates have an increased genome size and pulse field electrophoresis has revealed their different karyotypes. The results of electrophoretic karyotyping and measurement of the nuclear DNA contents of *Verticillium albo-atrum* hop isolates from various geographic origins will be reported.

68. A Chicken or Egg Dilemna: are Transposable Elements Drivers of Effector Birth and Diversification in *Leptosphaeria* Species? J. Grandaubert[1], M.H. Balesdent[1], H. Borhan[2] and T. Rouxel[1] [1] INRA-Bioger, Grignon, France ; [2] AAF Saskatoon, Canada

Leptosphaeria maculans and *L. biglobosa* are part of a species complex of fungal pathogens of crucifers. The genomes of two *L. maculans* 'brassicae' (Lmb) isolates (45.12 Mb, assembled into 76 scaffolds and 44.16 Mb assembled into 986 scaffolds, respectively) have an unusual bipartite structure – alternating distinct GC-equilibrated and AT-rich blocks of homogenous nucleotide composition. The AT-rich blocks comprise one third of the genome and contain effector genes and families of transposable elements (TEs), postulated to have recently invaded the genome, both of which are affected by Repeat Induced Point mutation. *In silico* comparison of the Lmb genomes with that of *L. maculans* 'lepidii' (31.53 Mb, assembled into 123 scaffolds) and *L. biglobosa* 'thlaspii' (32.10 Mb, assembled into 237 scaffolds), shows these species have a much more compact genome with a very low amount of TEs (<1%). In addition some recently expanded TE families are specific of *L. maculans* isolates. Compared to the Lmb genomes, less than 14% of the effector genes and 33% of other genes in AT-blocks are present in the two other genomes, suggesting TEs were key players in gene innovation and that the genome environment promoted rapid sequence diversification and selection of genes involved in pathogenicity.

69. Improved genome annotation for the mushroom *Coprinopsis cinerea*. CH Au[1], CK Cheng[1], SK Wilke[2], C Burns[3], ME Zolan[3], PJ Pukkila[2], HS Kwan[1] [1]The Chinese University of Hong Kong, Hong Kong, China [2]The University of North Carolina at Chapel Hill, NC, USA [3]Indiana University, IN, USA

The genome sequence of the model mushroom *Coprinopsis cinerea* recently published is an important resource in understanding the fungus. Gene models currently available are mainly derived from various computer prediction algorithms with help of EST sequences and manual curation. To examine the accuracy of the gene models, we analyzed 5' Serial Analysis of Gene Expression (5'SAGE) and microarray datasets we generated and the RNA-Seq dataset from Zemach *et al*. (*Science* 328:916-919). Gene model annotations of transcription start sites, 5' and 3' untranslated regions and exons are added or modified. Gene expression levels of different developmental stages are also incorporated. The new genome annotation datasets will be accessible through a web-based genome browser. This resource will be useful in *C. cinerea* functional genomics and comparative genomics of related fungal species. Further investigation of the datasets will also be presented.

70. Killing a nematode; Genome sequencing of the nematode-trapping fungus *M. haptotylum*. Tejashwari Meerupati, Karl-Magnus Andersson, Eva Friman, Anders Tunlid and Dag Ahren, Microbial Ecology, Lund University, Sweden. Email:dag.ahren@mbioekol.lu.se

Nematode-trapping fungi form specific morphological infection structures, called traps, to capture nematodes in soil. A successful infection includes adhesion, penetration of the nematode cuticle followed by digestion and assimilation of the nutrients. The nematode-trapping fungi are cosmopolitan and form a monophyletic clade belonging to the family of *Orbiliaceae, Ascomycota*. We have sequenced the genome of a nematode-trapping fungus, *Monacrosporium haptotylum*, which forms lollipop-like, single cell traps, called knobs. The *M. haptotylum*genome was sequenced to 20x coverage and is 39 Mb in size. We have identified 12 000 protein coding genes, including a large number of proteases. Subtilisin-like serine proteases belonged to one of the most expanded protein families which had 62 genes in *M. haptotylum* and have previously been shown to be involved in the infection of nematodes. In addition, several known fungal effector genes such as CFEM and Cerato-platanin genes have been identified. Proteomics analyses from the purified knobs and vegetatively growing mycelia show distinct differences in the proteome and provide important insights into the parasitic ability of the nematode-trapping fungi. Candidates including genes encoding subtiltisins and fungal effector genes have been selected for heterologous expression in the yeast *Pichia pastoris*.

71. Distribution and evolution of the fungal cellulose-binding module CBM1. Mathieu Larroque [1], Bernard Dumas [1] and Elodie Gaulin [1]. [1] UMR 5546 CNRS-Université Paul Sabatier, Castanet-Tolosan France, mathieu.larroque@scsv.ups-tlse.fr

The carbohydrate-binding module family 1 (CBM1) is a protein domain involved in cellulose binding and known to be widely spread in fungal plant cell wall degrading enzymes. Surprisingly, CBM1 were identified as part of the non-enzymatic CBEL and CBEL-like effectors of the oomycetal plant pathogens *Aphanomyces euteiches* and *Phytophthora sp* . To understand the presence of this protein domain in such evolutionary distant organisms, *in silico* analyses were engaged.649 CBM1 sequences among 130 species of sequenced microorganisms were collected from public databases. These sequences were aligned with ClustalW and the maximum-likehood phylogeny was determined with phyML leading to a CBM1 phylogenic tree drawn with Treedyn. CBM1 distribution was restricted to the oomycetes and fungi and related to their life style. The CBM1 phylogenic tree pointed out oomycetes-specific CBM1 patterns probably due to multiple events of CBM1 emergence in the oomycetes lineage. The study of the domain combinations associated to CBM1 showed that contrary to fungal CBM1, most of the oomycetal CBM1 were found in non-catalytic proteins. This may indicates different uses of CBM1 between oomycetes and fungi. Further details on this phylogenic study will be presented.

72. Complete genome sequence of *Fusarium asiaticum* strain SCK04. Haeyoung Jeong[1], Beom-Soon Choi[2], Ik-Young Choi[2], Theresa Lee[3], Sung-Hwan Yun[4]. [1]Industrial Biotechnology & Bioenergy Research Center, KRIBB, Daejeon 305-600, [2]NICEM, Seoul National University, Seoul 151-921, [3]National Academy of Agricultural Science, RDA, Suwon 441-707, [4]Department of Medical Biotechnology, Soonchunhyang University, Asan 336-745, Korea

The *Fusarium graminearum* (*Fg*) species complex, the causal agent of Fusarium head blight of small grain cereals, comprises at least 13 lineages, or phylogenetically distinct species. Among these, lineage 6 (*F. asiaticum*) is a major population of *Fg* complex recovered from rice in South Korea. We sequenced the genome of a representative strain (SCK04) of *F. asiaticum* using 454 pyrosequencing technology, and generated 2,926,888 shotgun reads and 1,039,879 paired end reads (~43X coverage). We assembled the sequence reads with Newbler assembler into 275 large contigs with a total length of 37.5Mb. After gap closure, we reconstructed five linear replicons that consist of four chromosomes, each corresponding to those of the previously sequenced *F. graminearum* strain PH-1 (*Fg* complex lineage 7) and a separated small segment (451kb). An initial set of 11,723 protein-coding genes were predicted using Augustus pre-trained on *F. gramineraum* gene set. Functional annotations of the predicted genes were carried out by hierarchical information transfer from the BLAST best hits using AutoFACT. Genome-wise comparison between SCK01 and PH-1 revealed a remarkable level of genomic synteny throughout the four chromosomes, but several rearrangements including inversions being located on chromosomes II and III in SCK04. Interestingly, the 451-kb fragment in SCK01 showed little sequence relatedness with the PH-1 genome.

73. Functional analysis of protein ubiquitination in the rice blast fungus *Magnaporthe oryzae*. Yeonyee Oh[1], Willian Frank[1], Angie Shows[1], Sang-Oh Han[2] and Ralph A. Dean[1]. [1] Dept. of Plant Pathology, Northe Carolina State University, Raleigh, NC. [2] Dept. of Medicine, Duke University, Durham, NC.

Rice blast is the most important disease of rice worldwide, and is caused by the filamentous ascomycete fungus, *Magnaporthe oryzae*. Protein ubiquitination, which is highly selective, regulates many important biological processes including cellular differentiation and pathogenesis in fungi. Gene expression analysis revealed that a number of genes associated with protein ubiquitination, including a polyubiqutin encoding gene, MGG_01282, were developmentally regulated during spore germination and appressorium formation. Inhibition of ubiquitin-mediated proteolysis using the 26S proteosome inhibitor, Bortezomib significantly attenuated spore germination and appressorium formation. In addition to a significant reduction in protein ubiquitination as determined by immuno blot assays, targeted gene deletion of MGG_01282 resulted in pleiotropic effects on M. oryzae including reduced growth and sporulation, reduced germination and appressorium formation and the inability to cause disease. Similar phenotypes were observed in the deletion mutant of MGG_13065, a SCF E3 ubiquitin ligase complex F-box protein. GFP subcellular localization studies revealed that polyubiquitin was highly expressed in intact spores and during appressorium development. Our study suggests that ubiquitination of target proteins plays an important role in nutrient assimilation, morphogenesis and pathogenicity of *M. oryzae*.

74. Silencing of velvet gene homolog suppresses cleistothecia formation in *Histoplasma capsulatum*. Meggan Laskowski-Peak[1], Ana M. Calvo[2], Jennifer Rohrssen[2], and A. George Smulian[1]. [1]University of Cincinnati and Cincinnati VA, Cincinnati, OH [2]Department of Biological Sciences, University of Northern Illinois, Dekalb, IL

Histoplasma capsulatum (Hc) is a dimorphic fungal pathogen, causing pulmonary disease that may progress to severe disseminated disease and death in immune-suppressed individuals. *Hc* loses mating ability over time in culture, limiting genetic tools available to study the organism. VeA, the velvet gene, regulates cleistothecial and conidial development in *Aspergillus nidulans*. Our work revealed that expression of the *Hc* veA homolog (VeA1) in the *A. nidulans* veA deletion strain rescues capacity to produce fruiting bodies. We hypothesized that VeA1 would play a role in regulation of mating in *Hc*. We silenced VeA1 in *Hc* strain UC26, which has re-gained the ability to form cleistothecia with mating competent clinical strains, to study the role of VeA1 in cleistothecia formation. The silencing vector was integrated by *Agrobacterium* transformation, and silencing was demonstrated by qRT-PCR. Mating structures formed in crosses between VA6 and the UC26 parent or control strains. No mating structures formed between VA6 and VeA1 silenced strains. These results indicate that VeA1 is necessary for cleistothecia formation, or is involved in regulation of cleistothecia formation in *Hc*. Further studies will determine whether overexpression of VeA1 enables mating in non-mating strains.

75. RIP and Transposable element evolution in 7 Sordariamycete genomes. Yi Zhou and Jason E Stajich. University of California, Riverside, CA. yi.zhou002@email.ucr.edu

RIP was first described in *Neurospora crassa* as a process that introduced C:G to T:A mutations in duplicated sequences during the sexual cycle and affecting tandem duplications greater than ~400bp or unlinked duplicates ~1kb. RIP-mutated sequences are frequent targets for methylation resulting in transcription silencing in Neurospora. In this way, RIP protects the genome from transposable element (TE) profileration. A commonly used method for detecting RIP relies on RIP indices. We found evidence of RIP using the RIP indices in 5 filamentous ascomycete fungi (*N. crassa*, *N. tetrasperma*, *N. discreta*, *Sporotrichum thermophile*, and *Thielavia terrestris*), but no evidence of RIP in *Chaetomium globosum* and *Sordaria macrosporus*. We developed a TE-annotation pipeline to look deeply into the TEs of these species, include homology-based methods (RepeatMasker), structure-based methods (LTR- harvest) and developed our own phylogeny-based methods. We found that *Gyspy* and *Copia* LTRs were the most abundant TEs. Our results presented two paradoxes: 1) *S.macrospora* and *C.globosum* have low percentage of interspersed repeats but lack evidence for RIP; 2) *S.thermophile* and *T.terrestris* show evidence for RIP but also have many repeats. We found that most TEs in these species longer than 1,500 bp are RIPed. Previous studies in *N. crassa* found that RIP requires ~ 80% identity over a length of ~ 400 bp, but we have found in other Sordariomycetes that TEs < 400bp are also RIPed.

76. Comparative Whole Deep Sequencing Transcriptome Analysis of the Dikaryotic Lifestyle and the Lignocellulolytic Strategies In the Model White Rot *Pleurotus ostreatus*. Antonio G. Pisabarro, Gúmer Pérez, Francisco Santoyo and Lucía Ramírez Genetics and Microbiology Research Group, Public University of Navarre, 31006 Pamplona, Spain. gpisabarro@unavarra.es

The new sequencing technologies are more powerful tools for transcriptome studies than the classic microarrays mainly because of their larger dynamic range of transcript abundance estimation. We have performed a whole transcriptome analysis (WTA) of the model white rot basidiomycete *Pleurotus ostreatus* using the Applied Biosystems Solid platform. The genome of *P. ostreatus* has been recently sequenced by the DOE Joint Genome Institute. The genomes of the two nuclei present in the dikaryotic strain N001 have been sequenced and assembled independently making *P. ostreatus* the first organism for which the two haplotypes have been effectively sequenced in a given individual. We have used these genome sequences as template for the annotation of the WTA data produced by monokaryons derived from each of the two N001 nuclei and for the N001 dikaryon itself, cultured under different conditions. This analysis has revealed the differences in the transcriptome landscape between monokaryons and dikaryons challenged by common environmental (culture) conditions. Besides that, the analysis also reveals the differential expression of genes involved in the degradation of lignocellulose by *P. ostreatus* and permits to compare the strategies used by this and other white and rot fungi whose genome is available.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

77. Resolving the Ascomycota tree of life through phylogenomic analyses of high-throughput sequence data. Cedar Hesse, Alex Boyd, Joseph Spatafora Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon

The Assembling the Fungal Tree of Life (AFTOL) project is a multi-laboratory research project focused on using genomic data to resolve ancient and problematic evolutionary relationships within the Kingdom Fungi. To this end, a set of 71 orthologous genes in Kingdom Fungi have been identified from published fungal genomes that will be used for phylogenetic placement of undersampled lineages of Fungi. In an effort to accelerate the data collection of the 71 target genes we have utilized the Illumina high-throughput sequencing platform to create rough draft *de novo* genome assemblies of numerous Ascomycota genomes. Our data collection includes a paired-end 80mer sequencing strategy that results in a median coverage of 20X – 120X. These data are assembled using Velvet 1.0.10 resulting in an average of 2,430 contigs greater than 300bp with an average N50 of 60.8kb. Using publicly available software (e.g., Augustus, HMMer 3) and custom developed bioinformatic tools (e.g., Hal) we are able to mine for phylogenetically informative target genes and produce genome-scale phylogenetic trees. Additionally, we are working to exploit the additional sequence data from the rough draft genome assemblies for more thorough phylogenomic analyses and gene content comparisons.

78. Comparative genomics of human fungal pathogens causing paracoccidioidomycosis. Christopher Desjardins, Jason Holder, Jonathan Goldberg, Sarah Young, Qiandong Zeng, Brian Haas, Bruce Birren, Christina Cuomo, and the Paracoccidioides Genome Consortium The Broad Institute, Cambridge, MA 02141 cdesjard@broadinstitute.org

Paracoccidioides brasiliensis is a dimorphic fungal pathogen and the causative agent of paracoccidioidomycosis, a human systemic mycosis endemic to Latin America. In order to better understand the biology of *Paracoccidioides* we sequenced the genomes of three strains: Pb01, Pb03, and Pb18. We also placed the Pb18 assembly on an optical map consisting of 5 chromosomes. Compared to their non-dimorphic relatives, *Paracoccidioides* and other dimorphic fungi encode a reduced repetoire of genes involved in carbohydrate and protein metabolism and synthesis of secondary metabolites. To compare genome content with phenotypic ability to utilize substrates for growth, we tested the related non-pathogenic *Uncinocarpus reesii* in metabolic assays. *U. reesii* displays broader and more effective growth on proteins than carbohydrates, which may predispose the dimorphic fungi to a pathogenic lifestyle. Furthermore, *Paracoccidioides* and other pathogenic dimorphs show expansions of the fungal-specific kinase family FunK1 and rapid evolution of transcription factors, suggesting specialized signaling and regulation potentially involved in dimorphism. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No.: HHSN2722009000018C.

79. Knockout and characterization of a polycomb group protein and proposed mating-type locus genes in Sclerotinia sclerotiorum. B. Doughan and J. Rollins. Plant Pathology Department University of Florida. Gainesville, Fl. 32607. USA.

Polycomb group proteins play important roles in animal and plant multicellular development through regulation of epigenetic memory. Functional polycomb group proteins have not been identified and characterized in fungi. We have identified a gene that encodes a protein sharing significant sequence similarity with the extra sex combs protein from *Drosophila melanogaster*. The proposed gene in *Sclerotinia sclerotiorum* (SS1G_13304.1) was identified as down regulated in apothecial stipes exposed to the light preceding the stipe to apothecial disc transition of *S. sclerotiorum*. This proposed sequence also contains a conserved WD 40 domain which is found in many eukaryotic regulatory genes. Gene replacement by homologous recombination failed to produce an observable phenotype in *S. sclerotiorum*. An over-expression construct using a stipe-tissue specific promoter has been used to transform the wild-type isolate and will be phenotypically characterized.. Another strategy to understanding the regulation of apothecial multicellular regulation is being pursued through functional characterization of the mating type genes in *S. sclerotiorum*. Gene deletion strategies are underway for the alpha domain sequences, the HMG domain sequence and two additional ORF present in the homothallic mating-type locus. Mutants will be characterized for mating type and apothecial development phenotypes.

80. Secretome discovery reveals lignocellulose degradation capacity of the ectomycorrhizal fungus *Paxillus involutus*. Doris Roth[1], François Rineau[2], Peter B. Olsen[3], Tomas Johansson[2], Andrea L. L. Vala[1], Morten N. Grell[1], Anders Tunlid[2], Lene Lange[1]. [1]Section for Sustainable Biotechnology, Aalborg University, Copenhagen, Danmark. [2]Department of Microbial Ecology, Lund University, Sweden. [3]Novozymes A/S, Bagsværd, Danmark. droth@bio.aau.dk

To improve our understanding of the role ectomycorrhizal fungi play in biomass conversion, we studied the transcriptome of *P. involutus* grown on glass beads in extract of soil organic matter. The mycelium was used for a cDNA library screened by Transposon-Assisted Signal Trapping (TAST*) for genes encoding secreted proteins. We identified 11 glycoside hydrolases (GH), none of them being cellulases of the GH families 6, 7 and 45, which constitute the well described enzymatic cellulose degradation system from numerous efficient cellulolytic fungi. In contrast, several predicted enzymes, namely a laccase and oxidoreductases possibly contribute to hydroxyl radical formation. The most abundant GH found was GH61, although typically described as accessory protein in the enzymatic cellulolytic apparatus. All in all, our results suggest that the cellulose degradation system of *P. involutus* resembles the brown rot fungi systems. In addition, GH61 apparently acts as accessory protein both in enzymatic and in radical-based cellulolysis. * Becker et al., J. Microbial Methods, 2004, 57(1), 123-33

81. Comparative study of Basidiomycete telomeres and subtelomeric regions. Gúmer Pérez, Antonio G. Pisabarro and Lucía Ramírez. Genetics and Microbiology Group. Public University of Navarre. 31006 Pamplona. Navarre. Spain. lramirez@unavarra.es

Telomeres and the subtelomeric regions are usually scarcely studied in genome sequence projects because of their repetitive nature and the occurrence of chromosomal rearrangements breaking down the synteny between these regions in closely related species. Fungal telomeres have been widely studied in Ascomycetes. In basidiomycetes, however, despite a number of fungal genomes (many of them corresponding to genera involved white biotechnology processes) have been sequenced, their telomeric and sub-telomeric regions are unknown. The study of these regions is of great importance since they have been described as harbouring secondary metabolite clusters. In our group, we have analysed the telomeric and subtelomeric regions in *Pleurotus ostreatus* using molecular and bioinformatics tools and, here, we perform a comparative study of these sequences in other sequenced basidiomycetes such as *Agaricus bisporus*, *Ceriporiopsis subvermispora*, *Heterobasidion annosum*, *Phanerochaete chrysosporium* and *Postia placenta* using a bioinformatics approach with the purpose of: i) determining the arrangement of genomes in putative linkage groups in species with no genetic maps available, ii) studying the synteny of the linkage groups arisen after informatics analysis of these genomes with the from *P. ostreatus*, and iii) determining the presence of similar genes the in subtelomeric regions of different genomes with different evolutionary history.

82. Genetic analysis of carotenoid biosynthesis and metabolism in a red basidiomycete yeast. Silvia C. Polaino and Alexander Idnurm. School of Biological Sciences, University of Missouri-Kansas City, 5100 Rockhill Road, Kansas City MO 64110, USA

Two major groups within the subphylum Pucciniomycotina, the rusts and red yeasts, derive their common names from the production of carotenoid pigments. Tools were developed for analysis of gene functions in the red yeast *Sporobolomyces* sp. strain IAM 13481, including targeted gene replacement and *Agrobacterium*-mediated transformation. Fifteen white T-DNA insertional mutants were isolated. The insertions lay in two adjacent genes that encode homologs of phytoene dehydrogenase and lycopene cyclase/phytoene synthase. Additional analysis revealed a third gene as part of the cluster, encoding a carotene oxygenase. The same three-gene cluster is also found in the genome sequence of another red yeast, *Rhodotorula glutinis* strain WP1. The organization of the cluster is identical to that found in *Gibberella fujikuroi* and related *Fusarium* species, which are ascomycetes. Phylogenetic analysis suggests that the gene cluster in the basidiomycete yeasts has arisen independently, rather than originate as a horizontal gene transfer event. The cluster may be maintained for the co-regulation of the biosynthesis of retinal, instead of carotenoids.

83. Evolution of the Respiro-fermentative Lifestyle in Ascomycota. Paul Muller Jr.1, Dawn A. Thompson1, and Aviv Regev1,2 1Broad institute of MIT and Harvard and 2Department of Biology, MIT, Cambridge, MA USA dawnt@broadinstitute.org

Central carbon metabolism (CCM) is a cornerstone metabolic system of yeast. Although CCM follows the same general outline in all yeasts, important biochemical, genetic and regulatory variations exist. For example, some species (e.g. S. cerevisiae) exhibit respiro-fermentative growth under aerobic conditions on glucose, whereas others (e.g. Kluyveromyces sp.) favor respiratory growth under similar conditions. A shift to a respiro-fermentative lifestyle has occurred more than once in the Ascomycota, most notably following the whole genome duplication event and once in the Schizosaccharomyces clade. The adaptive causes of this evolutionary shift in CCM are not clear. In order to understand the evolution and adaptive importance of the respiro-fermentative lifestyle we developed approaches that combine comparative and functional genomics to profile cladistically distinct species. In addition, we have made use of an ecologically relevant, highly fermentable, medium that may uncover phenotypes masked by more traditional laboratory medium. Preliminary data on biomass and ethanol accumulation, glucose consumption, and transcriptional response in species of the Saccharomyces, Kluyveromyces, and Schizosaccharomyces clades have yielded interesting results. As expected S. cerevisiae has a high rate of ethanol production – it was domesticated early in human history for this fact. However, surprisingly S. pombe exhibited a higher rate of ethanol production. By characterizing these responses in a variety of species with different lifestyles, we will distinguish their conserved parts from species-specific ones. Conservation across extensive phylogenetic distances will indicate likely functional essentiality, whereas divergence can be interpreted within the contexts of different ecological and historical constraints.

84. Using Next Generation sequencing to characterize a complex mutational event in Gibberella zeae. Brad Cavinder and Frances Trail, Michigan State University, East Lansing, MI cavinder@msu.edu; trail@msu.edu

We have generated a random insertional mutant of G. zeae lacking the ability to discharge ascospores. Southern hybridization failed to recognize the insertional plasmid confirming the existence of an untagged mutation. The mutation was genetically mapped to a 400 kb interval on Chromosome 2 in affected progeny using a tiered mapping strategy. To identify the site of mutation, the DNA of the mutant was sequenced using Illlumina technology. The sequence was assembled using Velvet and compared to the reference genome sequence. The region mapping to the mutation is next to a translocation of ~277 kb to Chromosome 1. We will present our strategy for identification of the mutated gene responsible for the discharge-minus phenotype.

85. Transcriptomic foundation of sexual development in Fusarium species. Usha Sikhakolli1, Jeffrey Townsend2 and Frances Trail1 1. Michigan State University 2. Yale University. Email: trail@msu.edu

The structure and morphology of fungal fruiting bodies have historically been the basis of taxonomic assignments and species definitions. However, recent phylogenetic analyses have resulted in a reorganization of the higher ascomycetes into monophyletic taxa that contain species with different fruiting body types. The implication is that large morphological changes may have repeatedly arisen from small genetic changes. We are using transcriptional profiling and detailed microscopy to illuminate the basis of morphological variation among species. Fruiting bodies formed by telemorphs of Fusarium spp. contain discrete tissues that develop sequentially as the fruit matures. Transcriptional profiling during the formation of these tissues has revealed shifts in transcription that result in morphological distinctions.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

86. Identification and potential function of natural antisense transcripts in the fungal plant pathogen *Ustilago maydis*. Michael E. Donaldson[1] and Barry J. Saville[1]. [1] Trent University, DNA Building, 2140 East Bank Dr., Peterborough, ON, K9J 7B8, Canada.

Natural antisense transcripts (NATs) corresponding to a number of open reading frames in the fungal plant pathogen *Ustilago maydis* were uncovered during the analysis of ESTs. Roles of NATs in regulating gene expression include: (1) transcriptional interference, (2) RNA masking, and (3) dsRNA-dependent mechanisms such as the broadly conserved RNA interference (RNAi) pathway. While plants, animals and most fungi contain functional RNAi machinery, phylogenetic and functional analyses have revealed that select yeast species and *U. maydis* do not. The role of NATs in *U. maydis* is currently unknown. We have characterized over 200 NATs by fully sequencing their corresponding antisense cDNAs. Using strand-specific RT-PCR, we determined that NATs are differentially expressed across a range of cell types, or expressed in a cell type-specific manner. The relationship between sense-antisense transcript pairs at four loci was examined in detail. In haploid cells, strand-specific quantitative-PCR, showed that at one of these four loci, the over-expression of antisense transcripts, whose expression naturally occurs in the dormant teliospore, increased the levels of its corresponding sense transcript. As a whole, experiments suggest that specific *U. maydis* antisense transcripts have the ability to stabilize sense transcripts. This action may be linked to the maintenance of mRNA integrity during teliospore dormancy and the controlled transition to actively translated mRNAs upon germination.

87. Identification and bioinformatic analysis of *Ustilago maydis* full-length cDNAs. Barry J. Saville[1], Colleen E. Doyle[1], Michael E. Donaldson[1], and Erin N. Morrison[1]. [1] Trent University, DNA Building, 2140 East Bank Dr., Peterborough, ON, K9J 7B8, Canada.

Ustilago maydis has been established as the model for basidiomycete biotrophic plant pathogenesis. A sub-library of cDNA clones representing full-length transcripts of 3058 genes was compiled from libraries used to create ESTs for annotation of the fully sequenced *U. maydis* genome. MIPS FunCat was used to assign gene function within the sub-library. Additional sequencing and genome comparisons allowed the identification of transcript domains and termini. For the genes represented in this library we have defined the 3' and 5' untranslated regions (UTRs), and isolated the sequences upstream of the initiation sites for transcription. Four datasets were used to identify conserved motifs with potential roles as 3' end processing signals, polyadenylation sites and signals involved in transcriptional control. The sequence elements we identified around the *U. maydis* poly(A) addition site show similarity to previously characterized yeast and plant signals. In the regions upstream of the start of transcription, a conserved sequence with similarity to the previously proposed *U. maydis* origin of replication was discovered. The association of gene function and conserved sequences enables further investigation of gene expression control in this model pathogen.

88. Domains of DNA methylation in *Coprinopsis cinerea* (*Coprinus cinereus*). Virginia K. Hench and Patricia J. Pukkila University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA ghench@med.unc.edu

Zemach et al. (Science 328:916-919, 2010) mapped the locations of 5-methylcytosine residues in the *C. cinerea* genome and proposed that repeated sequences, including transposable elements, are targets of DNA methylation in this species. Here we further characterize the domains in this genome in which over 25% of the CpG residues are methylated. These domains (14% of the genome) range in size from 0.3 to 100 kb, and include both repeated (802) and unique (335) genes. One domain on each chromosome includes methylated transposons that have been mapped to the cytological centromere (Stajich et al. PNAS 107:11889-11894, 2010). Since *C. cinerea* has an efficient machinery to detect and methylate tandemly repeated sequences (Freedman et al. Genetics 135:357-366, 1993), we examined the methylation status of several large repeated gene families. Most duplicated paralogs within families such as the Fun K1 kinases, cytochrome P450 genes, and hydrophobins are not methylated. We observe transcription of 54% of all methylated genes, including a DNA methyltransferase gene. Chromosome regions exhibiting elevated rates of meiotic recombination contain a 2.5 fold excess of methylated domains (X^2=116, $P < 0.0001$). These observations raise questions concerning factors that are required to establish and maintain repressive chromatin structures at methylated centromeres, transposon clusters, and genes, but not within other methylated regions. Supported in part by the HHMI through the Precollege and Undergraduate Science Education Program.

89. Identification of Polyketide Synthases in the *Ascochyta rabiei* genome. Javier A. Delgado[1], Richard P. Oliver[2], Judith Lichtenzveig[2], Francis Kessie[2], Ramisah Modh Shah[2], Samuel G. Markell[1] and Rubella S. Goswami[1] [1]Dept. of Plant Pathology, North Dakota State University, Fargo, North Dakota USA. [2]Dept. of Environment and Agriculture, Curtin University, Perth, Australia.

Polyketides are natural products synthesized by iterative type I polyketide synthases (PKSs) in fungi which often serve as pathogenicity or virulence factors. *Ascochyta rabiei* a devastating pathogen chickpea is known to produce at least one polyketide, solanapyrone, which has been associated with disease severity but not characterized. Our goal was to identify and characterize all the PKSs in *A. rabiei* from the recently sequenced draft genome. Contigs from the draft genome were analyzed with FGenesh and the predicted proteins were searched with a fungal PKS identification model specifically developed for predictions in fungi. This model consisted of profile hidden markov models based on the beta- ketoacyl and acyltransferase domains of fungal PKSs and had been validated on other ascocmycetes. The model predicted 11 PKSs in *Ascochyta rabiei*, 8 of which were determined to be reducing and 3 non-reducing. Nine of these PKSs were believed to be functional based in the presence of the acyl carrier protein domain. The predicted protein sequences of these PKSs ranged from 1857 to 4053 amino acids and included a potential solanapyrone synthase homolog. We also found that crude extracts from *Ascochyta rabiei* isolates grown under conditions inducing solanapyrone production show differential levels of phytotoxicity in *Chlamydomonas reinhardtii*. The expression of genes coding for the identified PKSs under different culture conditions and in-planta are currently being evaluated.

90. Comparative genomics and RNAi to find meiotic genes in *Coprinopsis cinerea.* Claire Burns[1], Erika Anderson[1], Allen C. Gathman[2], Walt W. Lilly[2], Jason E. Stajich[3], Patricia J. Pukkila[4], Miriam E. Zolan[1] [1]Indiana University, USA [2]South-East Missouri State University, USA [3]University of California, Riverside, USA [4]University of North Carolina, Chapel Hill, USA

Coprinopsis cinerea (also known as *Coprinus cinereus*) is a tractable basidiomycete that has natural synchrony of meiotic cell development, and is thus used as a model for meiotic studies. This unusual feature, not naturally observed in other organisms, allowed examination of a time course through *C. cinerea* meiotic development using microarrays. We compared gene expression patterns during meiosis in *C. cinerea*, *S. cerevisiae* and *S. pombe*, and noted that meiotic genes are more evolutionarily conserved in their expression patterns than non-meiotic genes[5]. Previously, novel meiotic genes have often been identified through conservation of meiotic gene expression within a species. We are now using a novel inter-species comparative approach for meiotic gene discovery in *C. cinerea*. In addition, we are using meiotic mutant transcriptional analysis to further refine the key genes involved in meiosis in *C. cinerea*. No basidiospores are produced if *C. cinerea* meiosis is defective, giving the mushroom cap a white appearance rather than the black, wild-type phenotype. Utilizing this easily screenable phenotype, and a meiosis-specific RNAi approach, we are beginning to screen putative meiotic genes identified though our comparative genomics, and progress will be presented. [5] Burns et al., Analysis of the Basidiomycete Coprinopsis cinerea Reveals Conservation of the Core Meiotic Expression Program over Half a Billion Years of Evolution. PLoS Genetics, 2010. 6(9) e1001135

91. Fungal Allergen Informatics. Mihaela Babiceanu[1], Ha X. Dang[1], Hirohito Kita[2], Barry Pryor[3]and Christopher B. Lawrence[1] [1]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, [2]Mayo Clinic Allergic Diseases Laboratory, Mayo Medical School, Rochester, MN, [3]Department of Plant Sciences, University of Arizona, Tuscon AZ

Human airways are constantly exposed to aeroallergens from fungi including, Cladosporium, Penicillium, Aspergillus and Alternaria. Sensitivity to *A. alternata* is a common cause not only of asthma, but also of upper respiratory disorders including chronic rhinosinusitis (CRS) and allergic rhinitis. In order to investigate the allergenic potential of *Alternaria spp.* and *Aspergillus fumigatus* we developed a bioinformatics approach utilizing fungal genome sequence information and a database of known allergenic proteins from a taxonomically diverse group of organisms. A set of 1407 known allergens was used to asses the level of identity with the predicted *A. brassicicola, A. alternata, A. fumigatus* genome-encoded proteomes and the hypothetical proteome encoded by a set of 10,000 *A. alternata* ESTs (spores germinating in the presence of sinus mucin). Using stringent cutoff conditions for assessing allergenic relevance we discovered that the number of homologues of known fungal allergens is larger than we anticipated (~75/species). Moreover, results of a specific query with 93 known fungal allergens showed that there were 70 homologs in the *A. alternata* ESTs suggesting high allergenic potential during spore germination. Finally, we identified homologues of diverse types or sources of allergens (insect, plant, venom, etc.) in fungal genomes.

92. Expanded Comparative Genomics at the *Aspergillus* and *Candida* Genome Databases. Jonathan Binkley[1], Martha B. Arnaud[1], Gustavo M. Cerqueira[2], Marcus C. Chibucos[2], Maria C. Costanzo[1], Jonathan Crabtree[2], Diane O. Inglis[1], Marek S. Skrzypek[1], Prachi Shah[1], Gail Binkley[1], Stuart Miyasato[1], Jennifer R. Wortman[2], and Gavin Sherlock[1] [1]Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA [2]Institute for Genomic Sciences, University of Maryland School of Medicine, Baltimore, MD, USA

The *Aspergillus* Genome Database (AspGD: www.aspgd.org) and *Candida* Genome Database (CGD: www.candidagenome.org) are curated, web-based genomics resources for researchers studying these diverse and clinically relevant groups of fungi. Initially focused on the well-characterized model organisms *Aspergillus nidulans* and *Candida albicans*, respectively, we recently redesigned AspGD and CGD to store and present curated genomic information for multiple species and strains. At AspGD, we currently include strains of *A. clavatus, A. flavus, A. fumigatus, A. niger, A. oryzae, A. terreus,* and *Neosartorya fischeri*. CGD will soon include strains of *C. dubliniensis, C. glabrata, C. guilliermondii, C. lusitaniae, C. parapsilosis, C. tropicalis, Debaryomyces hansenii,* and *Lodderomyces elongisporus.* In addition to providing sequence information, Gene Ontology annotation, and literature curation for these organisms, additional new features for both AspGD and CGD include: redesigned Locus Summary and Protein Information pages, with expanded links to orthologous loci across the different species; new Homologs pages, displaying multiple sequence alignments and phylogenetic trees for orthologous groups of genes; and a redesigned BLAST tool, allowing queries against any combination of database genomes. In the near future we plan to incorporate many additional genomes (including dozens of *A. fumigatus* clinical isolates) as well as high-throughput gene expression data from several different species. The expansion of AspGD and CGD to include multiple species will not only directly serve a wider base of researchers, but will also expand the power of comparative genomics for the entire fungal biology community.

93. The secretome of *Heterobasidion irregulare* growing on wood of spruce. Majcherczyk, Andrzej and Kües, Ursula Georg-August-Universität Göttingen, Germany

Heterobasidion species are severe pathogens in conifer plantations and natural forests mainly in Europe and the USA and cause root and butt rot in living trees. The fungi are white rots, degrading simultaneously or selectively lignin. The genome of the North American *Heterobasidion irregulare* was established by the JGI and the annotated genome can be used in studies of the fungal proteome. *H. irregulare* was grown in liquid medium with and without *Picea abies* wood. Freely secreted and hyphal sheath associated proteins analyzed by 2D-gel electrophoresis revealed a high diversity between wood supplemented and control cultures. Protein identification by ESI-LC-MS/MS was either performed on single protein spots form 2D-gels or by application of a shot-gun method on complex protein mixtures. Using a MASCOT database with the *H. irregulare* proteome as deduced from the fungal genome, in total 98 different secreted proteins have been identified. 58 proteins were present under both culture conditions and only six proteins were suppressed by wood supplementation. Addition of wood resulted in 36 new proteins secreted into the culture media. Redox-enzymes were represented by 21 proteins and most of them were induced by wood. Expression of laccases (except of one) and alcohol oxidases differed not between the two culture media. However, wood induced secretion of FAD-oxidoreductases and redox-enzymes with unknown function and furthermore induced secretion of specialized glycanases, lipases and proteases.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

94. *UBL1* of *Fusarium verticillioides* encodes a putative E3 ubiquitin ligase involved in growth, conidiation, virulence, amylolysis and secondary metabolism. Hirsch, RL; Ridenour JB; Bluhm BH. Department of Plant Pathology, University of Arkansas. bbluhm@uark.edu .

Fusarium verticillioides, a kernel rotting pathogen of maize, produces fumonisin mycotoxins linked to numerous human and animal toxicoses. Although amylolysis is critical for kernel colonization and fumonisin biosynthesis, the underlying molecular mechanisms are poorly understood. In this study, a collection of insertional mutants created by restriction enzyme mediated integration (REMI) was screened to identify strains impaired in amylolysis. In one mutant, the REMI cassette disrupted a gene (designated *UBL1*) predicted to encode a UBR-Box/RING domain E3 ubiquitin ligase. Disruption of *UBL1* in the wild-type strain caused pleiotropic defects in growth and development, including reduced conidiation, changes in hyphal morphology, and increased pigment production. Disruption of *UBL1* also significantly impaired kernel colonization, but the ratio of fumonisin B1 biosynthesis per unit growth was not significantly different than that of the wild-type strain. Somewhat surprisingly, disrupting the putative ortholog of *UBL1* in the closely related maize pathogen *Fusarium graminearum* did not have a pronounced impact on amylolysis. This study is one of the first to implicate a specific ubiquitin ligase in primary metabolism and pathogenesis among filamentous fungi, and provides a molecular foothold for the further dissection of amylolysis during kernel colonization.

95. Freely secreted and cell-wall-associated proteins from *Coprinopsis cinerea.* Majcherczyk, Andrzej, Güttel, Dorothea and Kües, Ursula Georg-August-Universität Göttingen, Germany

The fungal secretome is composed of different protein fractions. A large number of proteins are freely secreted to the hyphal environment whereas others are associated with hyphal cell wall structures. The cell wall of basidiomycetous fungi is composed by complex glycan network which is surrounded by an outer layer of less dense glycan structures called the hyphal sheath. We performed a study on the secretome of the model basidiomycete *Coprinopsis cinerea*, fractionated into the freely secreted proteins, proteins of the hyphal sheath and cell wall proteins extracted in series by NaCl, SDS and NaOH. 2-DE of the different fractions of proteins and identification of the proteins by a shotgun approach showed a significant difference between free and immobilized proteins. In total, we identified 116 unique proteins with partial overlapping between fractions. Glycoside hydrolases, peptidases and oxidoreductases dominated in the free secretome (in total 34 proteins) and in the hyphal sheath (in total 52 proteins). 65 different proteins were extracted from the cell walls, 53 of which were cell-wall-specific. Prominent enzymes were those involved in cell wall structuring, e.g. chitinases and mannosidases. The compartmentation suggests distinct biological functions for the protein in the different fractions. This work was supported in frame of a Common Lower-Saxony-Israel Project (ZN 2043) by the Ministry of Science and Culture in Hannover, Germany.

96. Explosive invasion of arbuscular mycorrhizal fungi by a mitochondrial group I intron Denis Beaudet[1], Jean-Claude Pasquet[1], B. Franz Lang[2] and Mohamed Hijri[1] [1]Institut de recherche en biologie végétale, University of Montreal, Montreal, Quebec, Canada. [2]Canadian Institute for Advanced Research, Centre Robert Cedergren, University of Montreal, Montreal, Quebec, Canada.

It has been previously showed that a group I intron has invaded the mitochondrial *cox1* gene of 48 angiosperm genera via 32 cross-species horizontal transfers. It has been suggested that this intron might have been transferred from fungi to plants and that Arbuscular Mycorrhizal Fungi (AMF) might be a plausible donor, due to their intimate interactions with plants and ubiquitous distribution. We succeeded to recover the intron sequences in 32 out of 44 AMF species member of 8 phylogenetically distinct families. The intron phylogeny showed that plants, AMF and *Rhyzopus oryzae* surprisingly clustered together which is incongruent with the organismal phylogeny. This suggests that this intron was in fact recently transferred to plants and AMF via a common fungal donnor.We also studied intraspecific polymorphism of the *cox1* intron using five isolates of the AMF *Glomus irregulare*. We found that its sequence wasn't complete and highly divergent in length in all of those isolates. Interestingly, for *Gigaspora rosea* and one isolate of *Glomus irregulare*there was no mtDNA copy of the intron. However, we managed to amplify a full length copy for both species. This strongly support that the intron has been transferred to the nuclear genome.

97. Seven genomes of plant-associated Clavicipitaceae. Christopher L. Schardl[1], C. Thomas Bullock[1], Patrick Calie[2], Mark L. Farman[1], Daniel R. Harris[1], Jerzy W. Jaromczyk[1], Jolanta Jaromczyk[1], Neil Moore[1], JinGe Liu[1], Jennifer S. Webb[1], Carolyn A. Young[3]. [1]University of Kentucky, Lexington, KY 40546, schardl@uky.edu [2]Eastern Kentucky University, Richmond, KY 40475, pat.calie@eku.edu [3]Samuel R. Noble Foundation, Ardmore, OK 73401, cayoung@noble.org

Clavicipitaceae function in plants as organ-specific replacement pathogens (e.g., *Claviceps fusiformis* and *Claviceps paspali*) or systemic parasites (e.g., *Aciculosporium take*), or as vertically transmitted symbionts of Poaceae (e.g., *Epichloë festucae* and *Neotyphodium coenophialum*) or Convolvulaceae (undescribed species). These fungi produce various alkaloids that are active against insect or vertebrate herbivores, and are presumed to play roles in protection of fungal propagules or host plant tissues. Genomes of the aforementioned species were sequenced, and clusters of alkaloid biosynthesis genes were identified. Complete gene clusters for indole-diterpene biosynthesis (*LTM*) were found in all except *N. coenophialum* and one of two sequenced *E. festucae* isolates, and genes for ergot alkaloid biosynthesis (*EAS*) were present in all except *A. take*. Genes for biosynthesis of lolines (*LOL*) and peramine (*PER*) were present only in *E. festucae* and *N. coenophialum*. Telomeric locations of *EAS*, *LOL*, and *LTM* may, in part, account for chemotypic variability of *Epichloë* species. Also variable among the Clavicipitaceae were orthologous genes for putative effector proteins, which we suggest may mediate host interactions and specificity.

98. Sequencing the Black Aspergilli species complex. Kuo, Alan[1*], Asaf Salamov[1], Scott Baker[2], and Igor Grigoriev[1]. [1]DOE Joint Genome Institute, CA, USA. [2]Pacific Northwest National Lab, WA, USA. [*]akuo@lbl.gov .

The ~15 members of the *Aspergillus* section *Nigri* species complex (the "Black Aspergilli") are significant as platforms for bioenergy and bioindustrial technology, as members of soil microbial communities and players in the global carbon cycle, and as food processing and spoilage agents and agricultural toxigens. Despite their utility and ubiquity, the morphological and metabolic distinctiveness of the complex's members, and thus their taxonomy, is poorly defined. We are using short read pyrosequencing technology (Roche/454 and Illumina/Solexa) to rapidly scale up genomic and transcriptomic analysis of this species complex. To date we predict 11197 genes in *Aspergillus niger*, 11624 genes in *A. carbonarius*, and 10845 genes in *A. aculeatus*. *A. aculeatus* is our most recent genome, and was assembled primarily from 454-sequenced reads and annotated with the aid of > 2 million 454 ESTs and > 300 million Solexa ESTs. To most effectively deploy these very large numbers of ESTs we developed 2 novel methods for clustering the ESTs into assemblies. We have also developed a pipeline to propose orthologies and paralogies among genes in the species complex. In the near future we will apply these methods to additional species of Black Aspergilli that are currently in our sequencing pipeline.

99. A genome comparison of pathogenic and non-pathogenic isolates of *Pyrenophora tritici-repentis.* V. Manning[1], L. Wilhelm[1], B. Dhillon[2], I. Pandelova[1], I. Grigoriev[3], L. Ma[4] & L. Ciuffetti[1] [1]Oregon State University, Corvallis, OR, 97331. [2]University of British Columbia, Vancouver, BC, Canada V6T 1Z4 [3] JGI, Walnut Creek, CA, 94598. [4] Broad Institute/MIT, Cambridge, MA, 02141.

The *P. tritici-repentis*-wheat pathosystem provides an excellent model for the study of gene-for-gene mediated susceptibility. Isolates that produce host-selective toxins (HSTs) are pathogenic on cultivars that are sensitive to these toxins and transformation of a non-pathogenic isolate with the gene(s) responsible for toxin production renders these isolates pathogenic on their respective hosts. However, two recent studies suggest that there are more differences between pathogens and non-pathogens than the presence/absence of HSTs. Therefore, we undertook a comparative analysis between pathogenic and non-pathogenic isolates utilizing the reference genome of a pathogenic isolate, Pt- 1C(BFP), EST libraries, and genome resequencing data. Significant differences between pathogenic and non-pathogenic isolates were found by mapping of ESTs and paired-end read data of resequenced isolates to the reference genome. A large number of SNPs, deletions, and indels were identified in the non-pathogenic resequenced isolate. A survey of repeat elements indicates that there are more DNA transposable elements in pathogens vs. the non-pathogen.

100. Hybrid Genome Sequencing of the Insect-Pathogen *Tolypocladium inflatum*. Kathryn E. Bushley and Joseph W. Spatafora, Oregon State University, Corvallis, OR

The recent genome sequences of several ascomycete fungi (e.g., *Grosmannia clavigera*, *Sordaria macrospora*) demonstrate the potential of hybrid approaches using both Illumina and Roche/454 next-generation sequencing technologies for de novo sequencing of fungi and other eukaryotic organisms. We have sequenced the genome of *Tolypocladium inflatum*, the producer of the immunosuppressant drug cyclosporin and one of the first insect-pathogenic fungi to be sequenced. Our hybrid sequencing approach has resulted in a draft assembly with 674 contigs, an N50 of 249 Kb, and a genome size of 30.8 Mb, which corresponds well with genome size predictions from published pulse-field gel electrophoresis studies. Ab initio annotations have predicted approximately 10,000 gene models. Comparisons of the gene space and transcriptome of *T. inflatum* with closely related plant pathogenic and endophytic fungi is providing insights into secondary metabolite arsenals of insect pathogens as well as shedding light on shifts in primary metabolism correlated with changes in nutritional mode within Hypocrealean fungi. In addition to the species-specific transposable element *Restless*, *T. inflatum* contains a unique repeat, the CPA element, which may contribute to genome plasticity in different strains. We will present data on the evolution and role of secondary metabolites in *T. inflatum* with an emphasis on nonribosomal peptide synthetases and the secondary metabolite gene cluster responsible for cyclosporin biosynthesis. We will also examine expansions and contractions of gene families involved in primary metabolism which may adapt *T. inflatum* for an insect pathogenic lifestyle.

101. Comparative genomics of xylose-fermenting fungi to enhance microbial biofuel production. Dana J. Wohlbach[1,2], Alan Kuo[3], Trey K. Sato[2], Katlyn M. Potts[1], Asaf Salamov[3], Kurt M. LaButti[3], Hui Sun[3], Alicia Clum[3], Jasmyn Pangilinan[3], Erika Lindquist[3], Susan Lucas[3], Alla Lapidus[3], Robert Zinkel[2], Kerrie W. Barry[3], Igor V. Grigoriev[3], Audrey P. Gasch[1,2] [1]University of Wisconsin-Madison, Madison, WI, [2]Great Lakes Bioenergy Research Center, Madison, WI, [3]US Department of Energy Joint Genome Institute, Walnut Creek, CA.

Cellulosic biomass is an abundant substrate for biofuel production; however, many microbes cannot natively metabolize pentose sugars within hemicellulose. Although engineered *Saccharomyces cerevisiae* can utilize the pentose xylose, the fermentative capacity pales in comparison to glucose, limiting the economic feasibility of industrial fermentations. To better understand xylose utilization for subsequent microbial engineering, we sequenced the genomes of two xylose-fermenting, beetle-associated fungi: *Spathaspora passalidarum* and *Candida tenuis*. To identify genes involved in xylose metabolism, we applied a comparative genomic approach across fourteen Ascomycete genomes, mapping phenotypes and genotypes onto the fungal phylogeny, and measured genomic expression across five Hemiascomycete species with different xylose consumption phenotypes. Together, this implicated many new genes and processes involved in xylose assimilation. Several of these genes significantly improved *S. cerevisiae* xylose utilization when engineered in this species. This work demonstrates the power of comparative methods in rapidly identifying key genes for biofuel production while reflecting on fungal ecology.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

102. MITEs in genomes of epichloid fungal endophytes. Damien Fleetwood[1,2], Anar Khan[1], Carolyn Young[3], Christopher Schardl[4], Richard Johnson[1] and Barry Scott[5] [1]AgResearch, New Zealand [2]School of Biological Sciences, University of Auckland, New Zealand [3]The Samuel Roberts Noble Foundation, Ardmore, Oklahoma [4]Department of Plant Pathology, University of Kentucky [5]Institute of Molecular BioSciences, Massey University, New Zealand

Miniature inverted-repeat transposable elements (MITEs) are abundant repeat elements in higher eukaryotes, however, this class of repeat has received less attention in fungi. Analysis of the genome sequence of the fungal endophyte *Epichloë festucae* revealed 13 degenerate MITE families that make up almost 1% of the *E. festucae* genome and relics of putative autonomous parent elements were identified for three families. Sequence and DNA hybridisation analysis suggest that all of the MITEs identified in the study were active early in the evolution of the epichloid lineage although are not found in other closely related genera. Analysis of MITE integration site preference showed that these elements have a target integration site preference for 5' genic regions of the *E. festucae* genome and are particularly enriched near genes for secondary metabolism. Copies of the EFT-3m/Toru element appear to have mediated recombination events that may have abolished synthesis of two fungal alkaloids in different strains. This work provides insight into the potentially large impact of MITEs on evolution of the epichloae and provides a foundation for analysis in other fungal genomes.

103. Challenges and approaches for sequencing and assembling the heterokaryotic genome of the soil fungus *Rhizoctonia solani.* Suman Pakala[1], Elizabeth Thomas[2], Marianela Rodriguez-Carres[2], Ralph Dean[2], David Schwartz[3], Shiguo Zhou[3], Rytas Vilgalys[4], Natalie Fedorova[1], William Nierman[1], and Marc A. Cubeta[2] [1]J. Craig Venter Institute, Rockville, MD, USA. [2]North Carolina State University, Raleigh, NC, USA. [3]University of Wisconsin, Madison, WI, USA. [4]Duke University, Durham, NC, USA. spakala@jcvi.org

The soil fungus *Rhizoctonia solani* (teleomorph *Thanatephorus cucmeris*) is an important pathogen of agricultural crops in the family Solanaceae that includes eggplant, pepper, potato, and tomato. To aid in understanding of the pathogenic and saprobic activity of the fungus, we are developing a high quality complete genome sequence of *Rhizoctonia solani* anastomosis group 3(AG-3), strain Rhs 1AP. Here we report the status of this project and discuss the challenges involved in sequencing a heterokaryotic genome. Specifically, we will present results (i)of a hybrid assembly of Sanger, 454 GS FLX and Illumina sequence reads and (ii)for optical map development which suggests that the genome size is ~86 Mb with at least 29 chromosomes. Analysis of optical map data also provided evidence for putative chromosomal translocations and trisomy. An experimental approach to obtain a reduced genome complement of the parental strain Rhs 1AP with SNP-based genetic markers to better facilitate the assembly and annotation of the genome sequence will also be discussed.

104. Empowering fungal genomics with sequencing. Carsten Russ, Terrance Shea, Brian Haas, Joshua Levin, Iain MacCallum, Broad Sequencing Platform, Louise Williams, Mike Ross, Patrick Cahill, Niall Lennon, Sarah Young, David Jaffe, Bruce Birren and Chad Nusbaum. carsten@broadinstitute.org. Broad Institute, 320 Charles Street, Cambridge, MA

Sequencing technologies continue to advance at a staggering pace, not only in terms of data yield (e.g. Illumina generates >30 Gb/day) but also in data types available (e.g. PacBio's single molecule >1Kb reads), thus empowering many applications. We will report on key technical advances, give examples and relate them to fungal genomics. Using improved laboratory and algorithmic methods, we can now generate high quality Illumina assemblies, for genomes from bacteria to mammals. Bacterial genomes frequently assemble into a single scaffold, and mammalian assemblies reach scaffold N50 sizes of 10-15Mb. We have begun deploying these methods on fungi with preliminary results for Schizosaccharomyces pombe, yielding scaffolds of 2.9Mb N50 size, with one (of 3) chromosome coming together in a single scaffold. RNA-seq has become a powerful tool for genome annotation and transcriptomics. Techniques for high quality strand- specific cDNA library construction and greatly improved algorithms for analysis and assembly of transcriptomes are now in place and regularly applied to fungal genomes (e.g. annotation of Schizosaccharomyces species and Salpingoeca rosetta). Sample input amount is critical to many applications including genome assembly. We are developing methods to enable rapid microbial assembly using either whole genome amplified DNA or sub microgram amounts of native DNA. Looking forward, we continue to seek out new technologies, such as Pacific Biosciences and Ion Torrent, to develop applications to leverage their strengths. For example, we are evaluating the power of Pacific Biosciences long and 'strobe' reads (with a 5-10kb span) in genome assembly and for detection of genome rearrangements.

105. Genome-wide screens using a natural product saponin identify three PDR pathway target genes, *PDR19, PDR20 and PDR21,* which influence lipid homeostasis and membrane permeability in *Saccharomyces cerevisiae.* Gary Franke, Daniel Chirinos, Virginia Aberdeen and Scott Erdman Dept. of Biology, Syracuse University seerdman@syr.edu

To investigate the mechanisms of action of an antimicrobial natural product saponin and to gain insights into lipid and membrane homeostasis in fungi, we carried out two genome-wide screens in yeast to identify genes involved in these cellular processes. A collection of 4,851 viable gene deletion strains was screened for growth rate on medium containing a triterpene glycoside (TTG) saponin. Deletant strains sensitive or resistant to TTGs were identified and collectively were found to be enriched for genes involved in several cellular processes, including lipid metabolism, cell wall assembly and toxin resistance. This screen identified many known, previously known and novel non-essential yeast genes whose absence affects growth under lipid and membrane disturbing conditions. A high copy plasmid suppression screen of one significantly TTG-sensitive mutant was also performed to learn more about TTG effects and potential mechanisms of resistance. This approach identified 11 different high-copy suppressors operating mainly through three pathways: vesicle trafficking, stress responses and the pleiotropic drug resistance (PDR) response. Analyses of the antifungal drug and chemical sensitivities of deletion strains for a subset of these high copy suppressors demonstrate them to be members of a novel group of PDR target genes, *PDR19, PDR20 and PDR21,* with specific roles in lipid and membrane homeostasis functions. Physiological studies of cells lacking these genes demonstrate their roles in influencing plasma membrane permeability in both normal and drug treated cells. Supported by NSF grants: SGER #0222591 and NSF #0315946

106. Withdrawn

107. Snapshot of the eukaryotic gene expression in muskoxen rumen- A metatranscriptomic approach. M. Qi[a],P. Wang[a,b],N. O'Toole[c],P. Barbozad[d],M. Leighand[d],B.Selinger[b],G. Butler[c],A. Tsang[c],T.McAllister[a], R. Forster[a] [a]Agriculture and Agri-Food Canada, Lethbridge Research Centre; [b]Department of Biological Science, University of Lethbridge;[c]Concordia University; [d]University of Alaska

A metatranscriptomic approach was used to study the functional and phylogenetic diversity of eukaryotes within the rumen of muskoxen (Ovibos moschatus). Polyadenylated RNA (messenger RNA) was sequenced on the Illumina Genome Analyzer II system and more than 2,800 M bp of sequences were obtained. Phylogenetic analysis revealed that rumen protozoa and rumen fungi were the dominant eukaryotes in the rumen. KOG database search showed that most of the assignable sequences belonged to the "Translation, ribosomal structure and biogenesis" cluster (48% of all sequences). Lignocellulose active enzyme encoding genes were identified from over 400,000 sequencing reads or 1100 contigs that were longer than 500 bp, including those rarely found in the previous gut metagenome studies. A fourth of the contigs exhibits less than 40% sequence identity to protein sequences in the Genbank non-redundant protein database, which are good candidate of novel glycoside hydrolases. The RNA-Seq data provided a snapshot of the expressed eukaryotic genes in muskoxen rumen and demonstrated an effective approach to identify functional eukaryotic genes from environmental samples.

108. Exploring gene origin in *Mycosphaerella populorum* via comparative genomic analyses. Braham Dhillon and Richard Hamelin. Department of Forest Sciences, University of British Columbia, 2424 Main Mall, Vancouver, BC V6T1Z4, Canada bdhillon@forestry.ubc.ca

Birth of new genes is an evolutionarily important phenomenon with implications on the phenotype and physiology of an organism. Although transposable elements have been thought to play a role in the birth of new genes, the exact mechanisms remain unknown. To investigate the origin of new genes, we performed a comparative genome wide scan between four plant pathogenic fungi from the genus *Mycosphaerella*. Preliminary results indicate the presence of 1,550 unique genes in *M. populorum*, 21 of which are present at syntenic breakpoints. Currently, we are investigating the origin and fate of these genes with respect to horizontal gene transfer, association with transposable elements and faster rate of accumulation of mutations in certain genes associated with pathogenicity.

109. Developing Parts List for Complex Fungal Systems from Biofuel Crop Ecosystem to Biorefinery. I gor Grigoriev US DOE Joint Genome Institute, Walnut Creek, CA, USA ivgrigoriev@lbl.gov

In contrast to fungi of medical importance, genome space of energy- and environment-associated fungi has been poorly sampled and became a focus of the Fungal Genomic Program at the US Department of Energy Joint Genome Institute. The key areas of the JGI Genomic Encyclopedia of Fungi include genomic exploration of fungi related to plant health (symbionts, pathogens, and biocontrol agents) and biorefinery processes (cellulose degradation, sugar fermentation, industrial hosts). Groups of fungi in each of these areas are sampled phylogenetically and sequenced genomes are analyzed as a group rather than individual genomes using comparative genomics approaches. Over 50 fungal genomes have been sequenced by JGI to date and released through MycoCosm (www.jgi.doe.gov/fungi), a fungal web-portal, which integrates sequence and functional data with genome analysis tools for user community. Sequence analysis empowered by functional characterization leads towards developing parts list for complex systems ranging from engineered ecosystems for biofuel crops to biorefineries. The former includes symbionts, pathogens, and biocontrol agents as parts of the system. The latter includes enzymes, pathways, and industrial hosts. Recent examples of parts suggested by comparative genomics and functional analysis in these areas will be presented.

110. Fruiting body development and transcriptomics in *Neurospora* species Nina Lehr, Zheng Wang, Francesc Lopez-Giraldez, Marta Farre, Frances Trail, and Jeffrey P. Townsend

Shifts in gene expression drive differentiation of tissues and the evolution of new morphologies in multicellular organisms. However, studies linking the evolution of gene expression and the evolution of development are difficult in complex organisms whose gene expression depends on environmental as well as genetic differences. We have carefully controlled the environment and developed novel techniques to examine microscopic phenotype and to assay genome- wide gene expression during perithecial development using next-generation sequencing in three species of *Neurospora*: the heterothallics *N. crassa* and *N. discreta*, and the pseudohomothallic *N. tetrasperma*. We have revealed elements of the underlying transcriptional program of fruiting body development. These developmental processes are fundamental to sexual reproduction, recombination, and to the adaptive dynamics of pathogens and hosts. This information, by comparison to other species such as Fusarium, will be used to estimate the ancestral evolutionary transitions that resulted in the shifts in morphology and ecology.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

111. Comparative Analysis of Dermatophyte Genomes. Diego A. Martinez[1], Sarah Young[1], Qiandong Zeng[1], Dermatophyte Genome Consortium, Bruce Birren[1], Ted White[2], and Christina Cuomo[1]. [1] Broad Institute of MIT and Harvard, Cambridge, MA [2] Seattle Biomedical Research Institute, Seattle, WA

Dermatophytes are fungi that cause superficial infections in humans and animals and are the most common fungal infectious agents on the planet, with current treatment costs exceeding one half-billion dollars annually. Despite the common occurrence of the disease little is known at the molecular level about the fungi that cause dermatomycosis. To unravel the genetic basis of this disease we have sequenced five dermatophyte genomes including the most common human dermatophyte, *Trychophyton rubrum*, along with related species that show differences in host preference and mating competence. These genomes were compared to outgroups including dimorphic fungi and Aspergilli to identify changes in content specific to the Dermatophytes as well as individual species. The Dermatophyte genomes are smaller than the outgroups, ranging from 22.5 to 24.1 Mb; the largest genome (*T. equinum*) has a larger amount of repetitive elements. Using comparative methods, we updated the annotations of the five species based on conservation of gene structures. Between 8,523 and 8,915 genes were predicted in each genome; this is slightly smaller than the outgroup fungi. The core gene set conserved in all five genomes includes nearly 80% of the protein coding genes. Based on whole genome alignments, the genomes are highly syntenic, with a small number of rearrangements between Trichophyton and Microsporon species. Further analysis of differences between genomes may help identify genes important for the specific adaptation of each species including potential virulence factors.

112. ChIP-Seq of Aspergillus niger: determining transcriptional regulation in key metabolic processes. Christina Sawchyn, Vanessa Blandford, Gregory Butler, Justin Powlowski, Adrian Tsang
Centre for Structural and Functional Genomics (CSFG), Department of Biology, Concordia University, Montreal, QC, Canada

Sequencing of immunoprecipitated chromatin DNA (ChIP-Seq) technology has enabled remarkable progress in the study of regulatory mechanisms at the transcriptional level. Made possible by the advent of next-generation sequencing, this technology combines chromatin immunoprecipitation and massively parallel sequencing to create a whole-genome picture of transcriptional regulation. Here we propose to use ChIP-seq technology to identify the downstream effectors of characterized transcription factors that have been shown to act in key metabolic and developmental processes in Aspergillus niger. Polyclonal antibodies have been raised against unique peptides contained in the sequences of the transcription factors AmyR and NirA, responsible for the amylolytic regulatory and nitrate assimilation pathways respectively, and have been tested for their specificity in immunoprecipitation assays for use in ChIP-seq. The results will be compared to the expression patterns of A. niger genes grown on various carbon sources and nitrogen conditions, as determined by RNA-blot analysis and RNA-Seq analysis. We expect the resulting data will help to elucidate the regulatory networks in A. niger as well as in other closely related fungal species.

Gene Regulation

113. Mating Type Specific Signaling Components of *Ustilago maydis*. Ben Lovely, Gregory E. Shaw, Kavita Burman Aulakh and Michael H. Perlin. University of Louisville, Department of Biology, Program on Disease Evolution, Louisville, Kentucky, USA

The phytopathogenic fungus *U. maydis* undergoes a dimorphic transition in which cell fusion and pathogenic development must occur for *U. maydis* to complete its lifecycle. Both cell fusion and pathogenicity are controlled by two loci, the *a* locus, encoding a pheromone and pheromone receptor, and the *b* locus, controlling pathogenic development. Mating of two cells of opposite mating type requires activation of the *a* locus via signal transduction through the mitogen-activated protein kinase (MAPK) pathway. The PAK-like Ste20 homologue, Smu1, is required for a normal response to pheromone via up-regulation of *mfa* expression. Deleting *smu1* reduced this up-regulation of *mfa* expression, with the effect more pronounced in the a2 mating background. A similar mating type specific defect also occurs with deletion of another PAK-like protein kinase involved in cytokinesis, Cla4. However, the effect was more pronounced in the a1 mating background. New evidence suggests that these mating type dependent defects in *smu1* and *cla4* deletion mutants extend to rates of growth as well, with *cla4* a1 mutants growing slower and *smu1* a2 mutants growing faster when compared to wild type. Also, yeast two hybrid analysis identified two potential Smu1 interacters, Rho1 and Hsl7, both of which exhibit mating type specific behaviors. Data suggest that while Rho1 is required for viability, when over-expressed, it also reduces the response to pheromone dramatically in the a2 mating background. Disruption of *hsl7*, causes cell elongation and reduction in the rate of growth, independent of mating background, yet only the deletion in the a1 background shows sensitivity to cell wall inhibitors. Thus, mating type dependent effects provide an interesting line of investigation into the overall control of mating and pathogenicity in *U. maydis*.

114. Yeast Rpn4 ortholog as a delta *veA* suppressor in *Aspergillus nidulans*. Jin Woo Bok[1], Dana J. Wohlbach[2], Yi-Ming Chiang[3], Clay C. Wang[3], Audrey Gasch[2] and Nancy P. Keller[1, 4] [1]Department of Medical Microbiology, [2]Department of Genetics, [4]Department of Bacteriology, University of Wisconsin-Madison, [3]Department of Pharmacology and Pharmaceutical Sciences, University of South California, USA

Recent identification of a heterotrimeric velvet complex VelB/VeA/LaeA in *Aspergillus nidulans* links light- responding development with secondary metabolism. To further understand how the velvet complex regulates secondary metabolism and development in this fungus, we screened both delta *laeA* and delta *veA* suppressors using an AMAI plasmid library of *A. nidulans*. We found several transformants that partially remediated the delta *veA* and delta *laeA* phenotypes to wild type. The sequence of the AMAI plasmid from one of transformants revealed a gene named ANID00709. This gene shows homology to yeast *rpn4* which encodes a transcriptional activator of the 26S proteasome. Rpn4 is involved in key regulatory roles in many aspects of cellular regulation, such as metabolic adaptation, cell cycle regulation, cell differentiation and protein degradation. The *A. nidulans rpn4* homolog, *rpnA*, rescued the wild-type phenotype in a yeast *rpn4* deletant. Over expression or deletion of *rpnA* affects secondary metabolite production, sporulation, and stress responses in *A. nidulans* as demonstrated by microarray, chemical and physiological analyses.

115. Histone biotinylation in *Candida albicans*. S.Hasim[1], Swetha Tati[1], Nandakumar Madayiputhiya[1], Kenneth W. Nickerson[1] [1]School of Biological Sciences, University of Nebraska-Lincoln,Nebraska

Candida albicans is an opportunistic fungal pathogen in humans. It is a polymorphic fungus: it can live as yeast, filamentous, or pseudohyphal forms. Hyphal and pseudohyphal forms of *C.albicans* are pathogenic. *C. albicans* is a natural biotin auxotroph. Biotin is required for cell growth and fatty acid metabolism. Biotin is also used as a cofactor for several carboxylases such as Acetyl-CoA, Pyruvate, and Methylcrotonyl–CoA carboxylase. In addition, we have discovered that biotin is also used to modify histones in *C. albicans*. During this study we observed two histones - H2B and H4 - being biotinylated in *C. albicans*. Residues K8, K11 in histone H4 and K17, K18 and K31 in histone H2B are biotin attachment sites in *C.albicans* and roughly 85% of those histone molecules are biotinylated. This histone biotinylation is reversible. We observed roughly equivalent levels of histone biotinylation under several growth conditions including aerobic and anaerobic growth and yeast and hyphal growth. So far the role of histone biotinylation in *C.albicans* is unknown. Techniques used to detect histone biotinylation in *C.albicans* did not detect any histone biotinylation in *Saccharomyces cerevisiae*.

116. Characterization of a Ste11 homologue MEEK gene, Cpm3k1-related mating response genes in *Cryphonectria parasitica*. Dae-Hyuk kim, Jin-Ah Park, Jung-Mi Kim, Kwang-Yeop Jang Institute of Molucular Biology and genetics, Center for Fungal pathogenesis, Chonbuk National University, Deokjin 664- 14, Jeonju, Jeonbuk, Korea 561-756

The gene *Cpm3k*1 of *Cryphonectria parasitica*, which encodes a MEEK homolog was cloned and characterized. The predicted protein sequence (CpM3K1) of the *Cpm3k*1 gene contains conserved MEKK domains, and sequence comparisons indicate that the cloned gene has high similarity to the known gene *Ste*11, which is involved in the mating pheromone response pathway in yeast. Gene replacement analysis revealed a high frequency of *Cpm3k*1 null mutants (6/54 stable transformants appeared to be the expected null-mutants). When compared with the wild-type parent strain, EP155/2, none of the *Cpm3k*1 null mutants showed any difference in terms of growth rate or pigmentation. However, when inoculated on agar substrate adjacent to chestnut twigs, the *Cpm3k*1 null mutants showed marked reductions in both the number and size of stroma on chestnut twigs, while the wild-type strain was able to effectively form abundant stromata. The pathogenicity test performed on the excised bark of a chestnut tree showed that compared with those of the wild-type and hypovirulent strains, *Cpm3k*1 null mutants produced necrotic areas of intermediate size. Disruption of the *Cpm3k*1 gene also resulted in defects in female fertility and down-regulation of transcripts for the mating pheromone precursor genes, *Mf2/1* and *Mf2/2*. In addition, transcription factors involved in the mating response pathway, such as *cpst12* and *pro1*, were down-regulated in the *Cpm3k*1 null mutants. Down-regulation of mating response marker genes, such as *Mf2/2*, *cpst12*, and *pro1*, was also observed in the mutant phenotype of *Cpmk2*, a mating response Fus3-like MAPK gene. These results indicated that the cloned *Cpm3k*1 gene is functionally involved in the mating response pathway and acts through downstream targets, including *Cpmk2*, *cpst12*, *pro1*, and *Mf2/2*. However, the mutant characteristics of the *Cpm3k*1 null mutant were fully phenocopied only in the *cpst12* null mutant, but not other null mutants of each component in the mating response pathway, suggesting a complex network in fungal signaling.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

117. The CarS carotene oxygenase links carotenoids, light and mating in *Phycomyces blakesleeanus* Tagua VG1, Ramírez-Medina H1, Martín-Domínguez R2, Eslava AP2, Cerdá-Olmedo E1, Corrochano LM1, Idnurm A3 1Dep Genet, Univ Sevilla, Sevilla, Spain; 2Dep Microbiol Genet/CIALE, Univ Salamanca, Salamanca, Spain; 3School Biol Sci, Univ Missouri, Kansas City, MO, USA E- mail: victor_tagua@us.es

The biosynthesis of beta-carotene and its regulation by light and sexual interaction has been investigated in the zygomycete fungus *Phycomyces blakesleeanus*. Mutations in the regulatory gene *carS* result in mycelia with large amounts of beta-carotene by disruption of the end-product inhibition. The gene *carS* is linked to the structural genes *carB* and *carRA*. From the genome sequence we have identified several candidate genes near the *carRA/carB* cluster. One of them encodes a protein similar to beta-carotene oxygenases, and we have found a mutation that replaced a Ser at position 433 by Leu in this gene in a *carS* strain. To confirm the identity of the putative *carS* gene we have sequenced this gene in 11 strains carrying six independent *carS* alleles. In all cases we have found single point mutations, including two mutations creating premature stop codons, in the putative beta-carotene oxygenase gene. In addition we sequenced this gene in five *carS* strains isolated after sexual crosses with *carS* parents. In all cases the *carS* progenies carried the same mutation as the *carS* parental strain. Since all the strains with different *carS* alleles had mutations in the beta-carotene oxygenase gene, and the mutation was passed to *carS* progenies after sexual crosses we conclude that this is the *carS* gene from *Phycomyces*.
The gene *carS* encodes a 628 aminoacid protein and contains two introns. The *carS* gene is repressed by light and it is activated by sexual interaction. We have observed that *carS* strains do not accumulate beta-carotene derivatives, including the sexual hormone trisporic acid. Our results show that CarS is a beta-carotene oxygenase required for the cleavage of beta-carotene to produce the sexual hormone of *Phycomyces*.

118. The bZip transcription factor RsmA regulates both sexual development and secondary metabolism. Wenbing Yin[1], Saori Amaike[2], Dana J. Wohlbach[4], Audrey P. Gasch[4], Yiming Chiang[5], Clay C. Wang[5], JinWoo Bok[1], Nancy P. Keller[1,3]. [1] Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, WI. [2]Plant Pathology Department, University of Wisconsin-Madison, WI. [3]Department of Bacteriology, University of Wisconsin-Madison, WI. [4]Department of Genetics, University of Wisconsin-Madison, WI. [5]Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, CA.

In a previous study, a putative bZIP protein, RsmA (remediation of secondary metabolism A), was identified by using a multicopy-suppressor genetics approach in Aspergillus nidulans. Overexpression of rsmA partially restores sterigmatocystin (ST) production in Velvet Complex mutants via transcriptional induction of ST gene expression. RsmA has high sequence homology to the Yap family of proteins in Saccharomyces cerevisiae, specifically Yap3. In yeast, the Yap family of transcriptional regulators plays an important role in response to various environmental stressors. We conducted a microarray experiment that further validated that RsmA transcriptionally regulates the ST gene cluster as well as sexual development. Several key genes (e.g. sdeA, mpkB and trxA) involving in the sexual development were up- regulated or down-regulated. Moreover, secondary metabolite extractions from OE RsmA contain seven additional compounds that have similar UV-Vis spectra with the known compounds, indicating very strong induction of the ST cluster and other metabolites in OE RsmA strains. Physiological experiments indicated that sexual stage was greatly delayed in OE RsmA strain. Taken together, these data suggest that RsmA regulates both secondary metabolism and sexual development and propose that regulation by RsmA functions through transcriptional control of crucial genes. Overexpression and deletion of these genes are currently underway to confirm these results.

119. ChIP sequencing reveal dual role for the transcription regulator Tri6 in the phytopathogen *Fusarium graminearum*. Charles G. Nasmith [1,2] Li Wang[1,2], Sean Walkowiak[1,2], Yunchen Gong[3], Winnie Leung[1], David S. Guttman[3], Gopal Subramaniam[1] [1]Agriculture and Agri-Food Canada, 960 carling Avenue, Ottawa, Ontario, Canada, K1A 0C6 [2]Contributed equally [3]CAGEF, University of Toronto, 25 Willcocks St. Toronto, Ontario, Canada, M5S 3B3 Email: subramaniamra@agr.gc.ca

The synthesis and accumulation of the trichothecene 15-acetyldioxynivalenol (15-ADON) is associated with Fusarium head blight (FHB) disease of cereal crops. Activation of the trichothecene gene cluster in the phytopathogen *Fusarium graminearum* requires the transcriptional regulator Tri6. Genome wide chromatin immunoprecipitation (ChIP) sequencing revealed targets of Tri6 both within and outside the trichothecene gene cluster. Bioinformatics analysis of the promoters of the targets established a consensus binding site for Tri6. The electro mobility shift analysis (EMSA) in addition to confirming the consensus binding site, but also identified another binding site specifically enriched in the promoters of the genes involved in secondary metabolism.

120. SrbA and its Role in Mediating Azole Drug Resistance in *Aspergillus fumigatus.* Sara J. Blosser, Sven D. Willger, Robert A. Cramer Jr Department of Immunology & Infectious Diseases, Montana State University, Bozeman, Montana, USA, sara.wezensky@msu.montana.edu

Deletion of the transcription factor SrbA results in complete growth inhibition under hypoxic conditions, avirulence in a murine model of Invasive Aspergillosis (IA), and increased sensitivity of *A. fumigatus* to triazoles. The purpose of this study is to investigate the mechanism and role of SrbA in mediating azole resistance. Azole drugs target ergosterol biosynthesis as their mechanism of action, and are the drug class of choice for treatment of IA. Erg11 (*cyp51*), the target of triazoles, is a 14-demethylase, and two functional copies (A/B) are encoded in the A. fumigatus genome. Transcript analysis shows down-regulation of Erg11A in the SrbA mutant, delta-srbA, suggesting regulation by this transcription factor. Induction of Erg11A in delta-srbA by regulatable promoter replacement restores wild-type levels of Erg11A, and ameliorates the azole sensitivity phenotype observed in delta-srbA. Repression of this construct restores azole sensitivity, demonstrating that Erg11A repression is partially or wholly responsible for the delta-srbA-azole phenotype. As SrbA appears to regulate Erg11A and several other key enzymes in ergosterol biosynthesis, understanding the regulon of SrbA could be vital in the development of higher-octane antifungals. Sterol intermediates in delta-srbA indicate a blockage in the Erg25 (C4-desaturase) enzymatic step, which is compounded in the Erg11A-induced strain. Studies investigating the induction of Erg25A with Erg11A in the delta-srbA background are underway.

121. Exploratory survey for potential transposable elements in *Aspergillus oryzae* by a stress-fluctuation cDNA browser. Hironobu Ogasawara[1], Saori Takahashi[1], and Katsuya Gomi[2] [1] Akita Res. Inst. Food and Brewing, Akita, Japan. [2] Graduate School of Agricultural Science, Tohoku University, Sendai, Japan. E-mail:hironobu@arif.pref.akita.jp

An active DNA transposon *Crawler* isolated from the genome of industrially important fungus *Aspergillus oryzae* transposes under extreme stress conditions [1]. The mRNA analysis of *Crawler* in the conidiospores revealed that cryptic splicing and premature polyadenylation of the mRNA occurred under the normal culture condition. The increasing in mature mRNA molecules was caused by stress treatment of $CuSO_4$ or heat shock, which could stimulate the transposition events allowing the full-length and active transposase to be produced. In this study, we carried out direct high-throughput paired-end RNA sequencing to construct a stress-fluctuation cDNA browser with DOGAN-DB to survey exogenous or transposon-like genes such as *Crawler* in *A. oryzae*. With comparison of expression pattern under extreme stress condition ($CuSO_4$) to the normal condition, several novel transcripts with open reading frames were identified in intergenic regions, where none of genes have been annotated in DOGAN-DB. Full length of DNA sequences encoding transposable elements were frequently identified. Among them, a novel transposable element homologous to *Tan1* from *A. niger* was identified and tentatively designated *AoTan1* that shows multiple characteristics of class II transposon. The elements are present as multiple copies in the genome of the RIB40 strain, suggesting that *AoTan1* is also expected to show a transposition activity. 1)H. Ogasawara *et al*. *Fungal Genet. Biol.* , **46**, 441-449 (2009)

122. Phenotypic analysis of *Neurospora crassa* chitinase knockout mutants. Georgios Tzelepis[1], Petter Melin[2], Jan Stenlid[1], Dan Funck Jensen[1] & Magnus Karlsson[1] [1]Department of Forest Mycology and Pathology, SLU, P.O. BOX 7026, SE-750 07 Uppsala, Sweden [2]Department of Microbiology, SLU, P.O. BOX 7025, SE-750 07, Uppsala, Sweden

Chitin is composed of β-1,4 N-acetyl-D-glucosamine units and it is an essential component of fungal cell walls. Chitinases are hydrolytic enzymes that play an important role in fungal biology e.g. in cell separation, spore germination, hyphal growth and mycoparasitism. Fungal chitinases belong to family 18, and are further divided into 3 clusters (A, B & C). The aim of this project is the phenotypic analysis of 12 *N. crassa* chitinase knockout mutants in order to investigate the function of these genes in connection to phylogeny. Selected parameters were tested, such as colony morphology in different media, sexual development, growth rate, sensitivity to stress conditions and fungal interactions. Mutant Δcht-12 displayed slower growth rate on carbon – rich media and on chitin compared to wt, while it grew faster in various cell wall stress conditions. In addition, Δcht-12 produced non-viable protoperithecia. Mutants Δcht-1 and Δcht-10 produced more protoperithecia compared to the wt when grown on synthetic crossing media. In dual cultures between mutants and *Fusarium sporotrichoides*, Δcht-10 produced higher protoperithecial numbers in the interaction zone than the wt mat-a strain. These results indicate the potential role of cluster B in cell wall structure and in nutrient acquisition.

123. Sex induced silencing defends the genome of *Cryptococcus neoformans* via RNAi. Xuying Wang[*1], Yen-Ping Hsueh[*2], Wenjun Li[1], Anna Floyd[1], Rebecca Skalsky[1], and Joseph Heitman[1] [1]Department of Molecular Genetics and Microbiology, Duke University Medical Center [2]Division of Biology, California Institute of Technology [*]These authors contributed equally.

Co-suppression is a silencing phenomenon triggered by the introduction of homologous DNA sequences into the genomes of organisms as diverse as plants, fungi, flies, and nematodes. Here we report sex induced silencing (SIS), which is triggered by tandem integration of a transgene array in the human fungal pathogen *Cryptococcus neoformans*. A *SXI2a-URA5* transgene array was found to be post-transcriptionally silenced during sexual reproduction. More than half of the progeny that inherited the *SXI2a-URA5* transgene became uracil auxotrophic due to silencing of the *URA5* gene. In vegetative mitotic growth, silencing of this transgene array occurred at ~250- fold lower frequency, indicating that silencing is induced during the sexual cycle. Central components of the RNAi pathway, including genes encoding Argonaute, Dicer, and an RNA-dependent RNA polymerase, are all required for both meiotic and mitotic transgene silencing. *URA5*-derived ~22-nt small RNAs accumulated in the silenced isolates, suggesting that SIS is mediated by RNAi via sequence-specific small RNAs. Through deep sequencing of the small RNA population in *C. neoformans*, we also identified abundant small RNAs mapping to repetitive transposable elements, and these small RNAs were absent in *rdp1* mutant strains. Furthermore, a group of retrotransposons was highly expressed during mating of *rdp1* mutant strains and an increased transposition/mutation rate was detected in their progeny, indicating that the RNAi pathway squelches transposon activity during the sexual cycle. Interestingly, Ago1, Dcr1, Dcr2, and Rdp1 are translationally induced in mating cells, and Ago1, Dcr1, and Dcr2 localize to P-bodies whereas Rdp1 appears to be nuclear, providing mechanistic insights into the elevated silencing efficiency during sexual reproduction. We hypothesize that the SIS RNAi pathway operates to defend the genome during sexual development.

124. Suppressor mutagenesis reveals EsaA as regulator of secondary metabolism. Alexandra A. Soukup, Joseph Strauss, and Nancy P. Keller Department of Genetics and Department of Medical Microbiology and Immunology, University of Wisconsin – Madison. 3455 Microbial Sciences 1550 Linden Drive Madison, WI 53706 soukup@wisc.edu

Regulation of secondary metabolite (SM) gene clusters in Aspergillus nidulans has been shown to occur through cluster specific transcription factors (AflR), or through global regulators of chromatin structure such as histone methyltransferases (ClrD, CclA), histone deacetylases (HdaA), or the putative histone methyltransferase LaeA. Deletion of *laeA* results in drastically decreased amounts of multiple secondary metabolites. A multi-copy suppressor screen for genes capable of returning SM to the Delta*laeA* mutant resulted in identification of the histone acetyltransferase AN10956.4, here referred to as *esaA*. Overexpression of *esaA* results in the induction of numerous SM clusters as well as sexual development. This effect is light dependent, emphasizing the importance of the velvet complex in regulating these stages of fungal development.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

125. Fungal Secondary Metabolism and its Impact on Ecological Interactions with Insects. Frank Kempken, Ulrike Fohgrub, Marko Rohlfs* Botanisches Institut und Botanischer Garten, Christian-Albrechts-Universität zu Kiel, Olshausenstraße 40, 24098 Kiel, Germany *Institute of Zoology and Anthropology, University of Göttingen

Using a combination of experimental ecology and functional genomic techniques the function of secondary metabolites (e.g. mycotoxine) as a chemical defence in insect-fungal interactions as well as the influence of these competitors at trophic interactions between insects ought to be investigated. This is based on previously published data (1-4). For our research the vinegar fly *Drosophila melanogaster* and its natural antagonist Aspergillus were used as a form of ecology model system. Microarrays of *Aspergillus nidulans* have been used to identify fungal target genes up- or down regulated when interacting with the antagonistic Drosophila larvae. Quantitative RT-PCR of RNA from *A. nidulans* confronted with *D. melanogaster* larvae indicates up regulation of the global regulator *laeA*, as well as *aflR*. In addition a number of other genes appear to be regulated under competing conditions. RNAi constructs of up regulated genes have been transformed into *A. nidulans* and are currently analyzed in competition experiments. In addition we are employing reporter gene constructs to monitor signal transduction in mycelia. (1) Rohlfs M, Trienens M, Fohgrub U, Kempken F (2009) In: The Mycota XV, pp131-151 (2) Kempken F, Rohlfs M (2009) Fungal Ecol, 3:107-114 (3) Rohlfs M (2005) Mycologia 97:996-1001 (4) Rohlfs M (2005) Frontiers in Zoology 2:2

126. Neurospora RCO-1/RCM-1 complex participates in photoadaptation. Carmen Ruger-Herreros[1], María Olmedo[2], Eva M. Luque[1], Gencer Sancar[3], Michael Brunner[3] and Luis M. Corrochano[1]. 1. Departamento de Genética, University of Sevilla, Spain. 2. Department of Chronobiology. University of Groningen. The Netherlands 3. Biochemie-Zentrum (BZH) Uniersity of Heidelberg, Germany

The activation of transcription by light of some *Neurospora* genes is transient, transcription of these responding genes ceases after the illumination time has been extended, and further incubation in the dark is required before they are again transcribed in response to light. This behavioral feature, photoadaptation, involves the transient binding of the photoresponsive White Collar Complex (WCC) to the promoters of light-regulated genes. We show that RCO-1 and RCM- 1, the *Neurospora* homologs of the components of the yeast Tup1–Ssn6 repressor complex, participate in photoadaptation. RCO-1 and RCM-1 are forming a complex that accumulates in the nuclei. Mutation in either *rco-1* or *rcm-1* result in high and sustained accumulation of mRNAs for *con-10* and other light-regulated genes after long exposures to light. Mutation in *rco-1* modifies the transient binding of the WCC to the light regulated promoters. Our results suggest that the Neurospora RCO-1/RCM-1 complex participates in the light-transduction pathway by repressing gene transcription after long exposures to light. Is the RCO-1/RCM-1 complex the proposed light- dependent repressor that modifies the activity of the WCC?

127. The decarboxylation of the weak-acid preservative, sorbic acid, requires a multi-enzyme complex in *Aspergillus niger*. Michaela Novodvorska, Andrew Plumridge, Malcolm Stratford, Petter Melin, Lee Shunburne, Paul S. Dyer, Jacques Stark, Hein Stam and David B. Archer School of Biology, University Park, University of Nottingham, Nottingham, NG7 2RD, UK.

Weak-acid preservatives are widely used to combat fungal spoilage of food and beverages. Certain spoilage moulds have the potential to completely degrade preservatives, such as sorbic acid, and thus enabling rapid spore outgrowth and formation of hyphae. Germinating conidia of *A. niger* are able to convert sorbic acid (2,4-hexadienoic acid) to the volatile compound 1,3-pentadiene. Two genes essential for this activity are strongly induced by sorbic acid; *padA1* encoding phenylacrylic acid decarboxylase; and *ohbA1* encoding o-hydroxybenzoic acid decarboxylase. Lying between these two genes is a putative transcription factor-encoding gene *sdrA*, encoding a putative sorbic acid decarboxylase regulator. Deletion of any of those three genes causes inability of mutant strains to decarboxylate sorbic acid. PadA1 and OhbA1 decarboxylases appear to interact in the decarboxylation process. We speculate that PadA1/OhbA1 create a multi-enzyme complex with a common active site. Essential features of a substrate for the decarboxylation complex include a carboxylic acid head-group, trans double-bonds at the 2 and 4 positions, and a carbon present at C6. More than 50 possible substrates have been discovered for this pleiotropic system and not all substrates serve as transcriptional inducers of the decarboxylation system. The *padA1*, *ohbA1* and *sdrA* genes are in close proximity to each other on chromosome 6 in the *A.niger* genome. Further bioinformatic analysis revealed conserved synteny at this locus in several *Aspergillus* species and other ascomycete fungi (including the yeast *Saccharomyces cerevisiae*) indicating clustering of metabolic function. Possible natural roles for these genes are discussed.

128. Modelling the transcription factor networks that control adaptation to hypoxia in *C. albicans*. André Nantel, Marco van het Hoog, Adnane Sellam and Malcolm Whiteway. Biotechnology Research Intitute, National Research Council, Montreal, QC, Canada. andre.nantel@nrc-cnrc.gc.ca

Our group has been exploiting ChIP-chip and microarray technologies, together with computer modeling, to provide a better understanding of select transcription factor (TF) networks that control environmental control in fungi. We measured the changes in transcriptional profiles that occur 5, 10, 20, 30, 45, 60, 75, 90 120 and 1440 min after the transfer of *C. albicans* to hypoxic growth conditions. Functional interpretation of these profiles was achieved using Gene Set Enrichment Analysis, a method that determines whether defined groups of genes exhibit a significant bias in their distribution within a ranked gene list. This required the production of a database of > 10,500 *C. albicans* gene sets taken from various databases of functional genome annotations, transcriptional profiles, TF-promoter interactions, as well as genetic and protein-protein interaction data. Visualization was facilitated by using the Cytoscape Enrichment-Map plug-in to produce networks in which mutually overlapping gene sets are clustered together. Hypoxia rapidly promotes strong changes in the transcript abundance of sugar metabolism genes. We used Network Component Analysis to evaluate the time-dependent changes in the activities of a dozen TFs (including Tye7p, Gal4p, Ndt80p and Sko1p), as well as to estimate their relative influence on 46 individual target gene promoters. These results were combined in a detailed and highly visual TF network model using BioTapestry.

129. Vader is suitable for transposon mediated mutagenesis in *Aspergillus niger* . Elkbir Hihlal, Ilka Braumann[1], Marco van den Berg[2], Frank Kempken*. Abteilung Botanische Genetik und Molekularbiologie, Botanisches Institut und Botanischer Garten, Olshausenstr. 40, 24098 Kiel, Germany; fkempken@bot.uni-kiel.de. Present address: Carlsberg Laboratory, Gamle Carlsberg Vej 10, 2500 Valby, Copenhagen, Denmark. DSM Biotechnology Center (699-0310), 2613 AX Delft, The Netherlands.

We have analyzed the transposon content in two fungal genomes, *A. niger* and *P. chryosogenum* (1). Among all transposons detected in *A. niger* CBS 513.88, only Vader elements appeared to be active (1). Strain CBS 513.88 is known to harbor 21 copies of the non-autonomous transposon Vader, which share a very high degree of sequence identity. We observed a Vader excision frequency of about 1 in $2.2x10^5$, spores. All colonies analyzed exhibited an excision event on the DNA level and Vader footprints were found. Employing TAIL-PCR the reintegration sites of 21 independent excision events were determined. All reintegration events occurred within or very close to genes. Vader appears to be a useful tool for transposon mutagenesis in *A. niger* (2). 1. Braumann I, van den Berg M, & Kempken F (2007) Transposons in biotechnologically relevant strains of *Aspergillus niger* and *Penicillium chrysogenum*. Fungal Genet Biol 44(12):1399-1414. 2. Hihlal E, Braumann I, Petersen N, van den Berg M, & Kempken F (2010) Vader is a suitable element for transposon mediated mutagenesis in *Aspergillus niger*. in preparation.

130. RRMA, an RNA binding protein involved in regulated mRNA degradation. Kinga Krol[1], Igor Y. Morozov[2], Piotr Weglenski[1,3], Yongxiang Fang[2], Massimo Reverberi[4], Mark X. Caddick[2], Agnieszka Dzikowska[1,3] 1. Institute of Genetics and Biotechnology, University of Warsaw, Poland 2. Institute of Integrative Biology, Liverpool University, UK 3. Institute of Biochemistry and Biophysics, Polish Academy of Science 4. Department of Plant Biology, Universita Sapienza, Rome, Italy kinga@igib.uw.edu.pl, a.dzikowska@wp.pl

RRMA is the RNA binding protein involved in posttranscriptional regulation of gene expression in Aspergillus nidulans. rrmA gene was identified as a suppressor of mutations in arginine/proline catabolic pathway. Independently RRMA protein was shown to bind to the 3'UTR of areA transcript (nitrogen positive regulator). Delta rrmA mutation results in slow growth phenotype and higher sensitivity to oxidative stress. Analysis of main antioxidant enzymes revealed different activity pattern during early development stages and under oxidative stress in delta rrmA strain comparing to the control strain. Microarrays analysis has shown that delta rrmA mutation results in changed stability of specific transcripts under conditions of oxidative stress and nitrogen starvation, e.g. the stability of deoxyhypusine synthase mRNA is decreased in delta rrmA. The enzyme is responsible for modification of eukaryotic initiation factor 5A (eIF5A) implicated in stress- induced translational repression. Our results indicate that RRMA plays important role in the mechanism of regulated degradation of specific mRNAs in response to specific signals.

131. Activity of AREA and AREB under different carbon and nitrogen regimes. Maria Macios[1], Piotr Wêgleñski[1,2], Agnieszka Dzikowska[1,2] [1]Institute of Genetics and Biotechnology, University of Warsaw, Poland [2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland adzik@ibb.waw.pl; marym@op.pl

Nitrogen metabolite repression modulates the expression of target genes participating in utilization of alternative nitrogen sources, resulting in transcription only when glutamine or ammonium levels are limiting. In *Aspergillus nidulans* this regulatory mechanism depends on a positively acting regulator AREA. At variance with other pathways regulated by AREA, this factor functions as a repressor of arginine catabolism genes, acting concertedly with the other GATA factor: AREB. The activities of AREA and AREB are differentially regulated by the carbon regime: AREA being necessary for the ammonium repression of *agaA* and *otaA* under carbon repressing conditions, while AREB is primarily involved under carbon-limiting conditions. The ability of both AREA and AREB to sense the status of carbon metabolism is most probably dependent on NMRA, and not on the transcription factor CREA, which mediates general carbon catabolite repression is *A. nidulans*. Our current research is focused on investigation of the hypothesis of AREA/AREB cooperation under different carbon and nitrogen regime.

132. Identification of a novel regulator controlling the cellulase gene induction in *Aspergillus aculeatus*. Emi Kunitake, Shuji Tani, Jun-ichi Sumitani, and Takashi Kawaguchi. Graduate School of Life & Environmental Sciences, Osaka Prefecture University, Osaka, Japan.

Aspergillus aculeatus secretes superior cellulolytic and hemicellulolytic enzymes on saccharification. It has been revealed that a Zn(II)2Cys6 transcription factor, XlnR, positively regulated those gene expressions in *Aspergillus*. However, we found that the *xlnR* gene disruption did not affect the transcription levels of cellobiohydrolase I (*cbhI*) and carboxymethylcellulase 2 (*cmc2*) genes in *A. aculeatus*. These data suggests that those genes are regulated by namely XlnR-independent signaling pathway. To identify regulator(s) participating in the regulation network, we established a positive screening system using a PcbhI-pyrG reporter fusion. Mutations were introduced by T-DNA insertion utilizing *Agrobacterium tumefaciens*-mediated transformation system, and which enabled to rescue the franks of T-DNA by PCR-based technique. Transformants were selected by indexing 5-FOA resistance and cellulose utilization deficiency, and then further analyzed on expression level of the *cbhI* and *cmc2* by RT-PCR. One out of 6,000 transformants was, so far, identified as a cellulase induction-deficient mutant. The T-DNA was verified to insert into near 5• f-end of ORF encoding putative a Zn(II)2Cys6 transcription factor, namely factor A. The factor A gene disruption by homologous recombination resulted in reduction of the *cbhI* and *cmc2* transcripts, demonstrating that Factor A participated in the XlnR-independent signaling pathway.

133. Two *Ustilago maydis* homologs of *Aspergillus veA* are required for normal disease development in maize. Brijesh Karakkat and Sarah F. Covert, Department of Plant Pathology and Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602 U.S.A.

Members of the fungal-specific *velvetA (veA)* gene family affect spore production in saprobic Ascomycetes. However, the functions of similar proteins in Basidiomycetes have not been established. We predicted that *veA* gene homologs in the basidiomycete plant pathogen *Ustilago maydis* might regulate spore formation, spore viability, and disease progression. To pursue these hypotheses, three *U. maydis* genes *Um00893, Um04203* and *Um01146* were identified by BLAST searches as *veA* family members. Using a gene replacement strategy, deletion mutants were made in all three genes. None of the mutants showed any phenotypic alteration during yeast-like, *in vitro* growth. However, the *Um00893* mutants failed to induce gall or teliospore formation in maize. Chlorazol staining of leaves infected with *Um00893* mutants revealed that the mutant hyphae did not proliferate normally during the early stages of infection. The *Um01146* mutants were able to induce galls, but were reduced in virulence. The *Um04203* mutants were not affected in disease progression. These data indicate that two *veA* family members in *U. maydis* are essential for normal disease development in maize.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

134. Role of bZIP transcription factors in the asexual development of the plant pathogen *Phytophthora infestans.* Heber Gamboa-Melendez and Howard S. Judelson Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA

Phytophthora infestans is the causal agent of late blight in potato and tomato crops. Plant infection by this pathogen can involve two modes of asexual development. One involves entry of hyphae from germinated sporangia through wounds or natural openings. In addition, sporangia can produce zoospores which encyst and germinate to form infection structures called appressoria that helps to penetrate the host. Gene expression in all developmental stages must be tightly regulated to allow a successful asexual cycle and infection. bZIP transcription factor family have essential roles in development in many eukaryotes. Identification and functional characterization of bZIP transcription factors in P. infestans will contribute to our understanding of their role in the asexual development and pathogenicity of this oomycete. Bioinformatic studies showed that P. infestans contains around 20 bZIP TFs. qRT-PCR showed that most were differentially expressed at different developmental stages. Overexpression and RNAi-based gene silencing methods, using both constitutive promoters and a chemically-inducible gene expression system based on the ecdysone receptor, are currently being used to study gene function. So far, a monopartite and two- hybrid ecdysone inducible system were tested. The monopartite version showed high expression levels of the reporter but high background without the inducer. The two-hybrid system showed lower expression levels but displayed a very low background in the absence of inducer.

135. Regulation of primary and secondary sporulation by Myb transcription factors in the oomycete *Phytophthora infestans* Qijun Xiang and Howard Judelson, Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521 USA

In our previous studies, a Myb transcription factor binding site was determined to be involved in regulating sporulation- specific gene expression, and genome-wide analysis identified several distinct phylogenetic groups of Myb transcription factors in the genome. In this study, the regulatory roles of these Myb proteins in sporulation are examined by gene overexpression. The phenotypes induced by overexpression distinguish two kinds of sporulation as follows:1)Primary sporulation. This is the canonical developmental process during which sporangia are produced from vegetative mycelia. The overexpression of *Myb2R4*, a R2R3 Myb gene, increased sporangia production significantly. qRT-PCR shows that a few other Myb genes are up-regulated by *Myb2R4* overexpression, suggesting some Myb genes form a regulatory cascade. 2) Secondary sporulation. This is a phenotype in which a sporangium germinates and then directly produces a new spore without an obvious vegetative growth phase. The overexpression of *Myb3R6*, an oomycete- specific R1R2R3 Myb gene, induced a high percentage of secondary spore production. The overexpression of *Myb2R5*, a R2R3 Myb gene, increased secondary sporulation significantly under inducing condition. Our assumption is that the overexpression of *Myb3R6* or *Myb2R5* reduces the dormancy of sporangia; however, the spores undergo secondary sporulation when the conditions do not favor direct germination.

136. Transcription modulation and mRNA processing of genes involved in the pH-sensing in *Aspergillus nidulans* are nutrient-dependent Trevisan, GL[1]; Martinez Rossi, NM[2]; Rossi, A [1] [1]Department of Biochemistry and Immunology, Faculty of Medicine of Ribeirão Preto, University of São Paulo. [2]Department of Genetics, Faculty of Medicine of Ribeirão Preto, University of São Paulo e-mail: gltrevisa@gmail.com

The fungal ability to regulate gene expression is essential for their development and adaptation to environmental changes. These adaptive responses include the nuclear RNA processing and a number of regulatory steps that can affect, for instance, the export, localization, translation, and stability of the transcripts. The inorganic phosphate (Pi) acquisition system (*PHO*) and the culture pH sensing (*PacC* signaling transduction pathway) are key metabolic circuits for the regulation of RNA processing. The expression of *pacC* and *palB* genes were evaluated in the control and mutant palB7 strains of *A. nidulans* grown in minimal medium (MM) or YAG supplemented with low (0.1 mM) or high (11 mM) Pi, pH 5.0 or 8.0. We observed that the level of *pacC* gene transcripts is responsive to Pi concentration changes and culture medium composition. Gene *palB* shows a different transcription profile: the balance between the processed and non-processed mRNA forms (with the intron retained) is dependent on both Pi concentration and the pH of the culture medium. These results reveal novel aspects of the pH-sensing network in *A. nidulans*. Financial support: FAPESP, CNPq, CAPES, and FAEPA.

137. LaeA control of velvet family regulatory proteins for light-dependent development and fungal cell-type specificity. Özlem Sarikaya Bayram[1], Özgür Bayram[1], Oliver Valerius[1], Hee Soo Park[2], Stefan Irniger[1], Jennifer Gerke[1], Min Ni[2], Kap-Hoon Han[3], Jae-Hyuk Yu[2] and Gerhard H. Braus[1] [1]Dept of Molecular Microbiology & Genetics, Georg August University, Göttingen, Germany [2]University of Wisconsin, Madison, USA [3] Woosuk University, Wanju, Korea

VeA is the founding member of the velvet superfamily of fungal regulatory proteins involved in light response and coordinates sexual reproduction and secondary metabolism in *Aspergillus nidulans*. In the dark, VeA bridges VelB and LaeA to form the VelB-VeA-LaeA (velvet) complex. Here we show that VelB forms a second light-regulated developmental complex with VosA. LaeA plays a key role not only in secondary metabolism but also in directing formation of the VelB-VosA and VelB-VeA-LaeA complexes. LaeA controls VeA modification and protein levels and possesses additional developmental functions. The laeA null mutant results in constitutive sexual differentiation, indicating that LaeA plays a pivotal role in inhibiting sexual development in response to light. Moreover, the absence of LaeA results in the formation of significantly smaller fruiting bodies. This is due to the lack of a specific globose cell type (Hülle cells), which nurse the young fruiting body during development. This suggests that LaeA controls Hülle cells. In summary, LaeA plays a dynamic role in fungal morphological and chemical development, controls expression, interactions and modification of the velvet regulators.

138. Analysis of the interplay between the GATA transcription factors AreA and AreB in *Fusarium fujikuroi* and identification of common and differential target genes. C.B. Michielse, P. Rengers, C. Bömke, P. Wiemann, B. Tudzynski Institute of Biology and Biotechnology of Plants, Westfälische Wilhelms-University Münster, Schlossgarten 3, D-48149 Münster, Germany. c.b.michielse@uni-muenster.de

In many fungi, including *Fusarium fujikuroi*, AreA, belonging to the GATA family of DNA-binding proteins, is the global regulator of nitrogen metabolite repression. In addition, in *F. fujikuroi* AreA is also a positive regulator for production of the secondary metabolite gibberellin. A second GATA transcription factor, AreB, first identified in *Aspergillus nidulans*, was shown to negatively regulate AreA-dependent gene expression under nitrogen starvation (1). To gain insight in the role of AreB and its putative influence on AreA-dependent gene expression in *F. fujikuroi areA/areB* single and double deletion mutants were generated and the growth phenotypes of each mutant was extensively compared. In addition, cellular localization of both proteins under nitrogen surplus and starvation conditions was determined. Finally, common and differential AreA and AreB target genes were identified using a combination of microarray and northern analysis. 1) Wong KH, Hynes MJ, Todd RB, Davis MA (2009) Deletion and overexpression of the *Aspergillus nidulans* GATA factor AreB reveals unexpected pleiotropy. Microbiology 155:3868-80.

139. Expression pattern of secondary metabolic genes under various culture conditions. M. Umemura[1*], H. Koike[1], M. Sano[2], N. Yamane[1], T. Toda[1], Y. Terabayashi[1], Y. Osawa[1], K. Abe[3], S. Ohashi[2], and M. Machida[1] [1]Natl. Inst. Adv. Indust. Sci. Tech., [2]Kanazawa Inst. Tech., [3]Tohoku Univ., Japan. [*]umemura-m@aist.go.jp

Fungi produce secondary metabolites which can be good candidates for bioactive agents. It is difficult, however, to obtain the metabolites from cell culture as fungi produce them only under certain particular conditions. If we could design culture condition under that desirable secondary metabolites are produced, such knowledge should lead to discovery of novel secondary metabolites. Toward this goal, we analyzed gene expression distribution of *Aspergillus oryzae* under more than 200 culture conditions using DNA microarray. We used *A. oryzae* as a model although it produces bare secondary metabolites. The culture conditions are mainly classified into three categories: 1. nutrition, 2. time, and 3. chemical addition such as antifungal agents. Each category has sub-classes. Combined with another technology of ours to predict fungal secondary metabolic genes, expression distribution of secondary metabolic genes under each condition was hierarchically clustered. Interestingly, the grouped cluster of gene expression distribution overlaps with the class of culture condition to some extent. The same tendency was observed when selecting the genes considered to concern fatty acid synthesis. This result will lead to the clue to the conditions under that fungi tend to produce more secondary metabolites.

140. The osmosensing signal transduction pathway from *Botrytis cinerea* regulates cell wall integrity and MAP kinase pathways control melanin biosynthesis with influence of light. Weiwei LIU[1] , Marie-Christine SOULIE[2], Claude PERRINO[2], and Sabine FILLINGER[3] [1]Institute for Research in Immunology and Cancer, Université de Montréal, Canada; [2]UMR 217 Interactions Plantes-Pathogènes, INRA-Agro ParisTech-Université Paris VI, Paris, France; [3]BIOGER CPP INRA Versailles-Grignon, France

Mitogen activated protein kinase (MAPK) signal transduction pathways are ubiquitous among eukaryotic organisms with evolutionary conserved modules. Although generally classified as osmotic and cell wall integrity pathways, functional divergences have been observed for HOG1- and SLT2-related MAPK pathways. Here we show that the osmotic signal transduction cascade is involved in cell wall integrity in the phytopathogenic ascomycete *Botrytis cinerea*. The deletion mutants of the upstream histidine kinase (HK) Bos1 and of the MAPK Sak1 showed modified tolerance to cell wall degrading enzymes and cell wall interfering agents, as well as increased staining of ß1-3 glucan and chitin compared to the wild-type. The Sak1 MAPK was phosphorylated upon cell wall challenging. Our analysis also revealed that Sak1 interferes with the phosphorylation status of the SLT2 type MAPK Bmp3 upon oxidative stress hinting to cross talk between both MAPK pathways. We observed differences in mycelial pigmentation for all three MAPK mutants of *B. cinerea* according to light exposure. Most but not all differences can be explained by differential expression of melanin biosynthesis genes. Melanin biosynthesis is differentially regulated by all three MAPKs in *B. cinerea* after the perception of light, probably equilibrating melanin biosynthesis in the dark and bright. The role of the upstream HK Bos1 in the melanin regulatory process is yet unclear.

141. Role of MAP kinases pathways in the infection process of the wheat pathogen *Mycosphaerella graminicola.* Elisabetta Marchegiani, Sian Deller, Thierry Marcel, Marc-Henri Lebrun UR 1290 BIOGER-CPP, INRA, Thiverval-Grignon, France

Mitogen-activated protein kinases (MAPKs), are essential components of evolutionary conserved signaling pathways in eukaryotic cells. *Mycosphaerella graminicola* a worldwide pathogen of wheat causing *Septoria tritici* leaf blotch (STB), has three MAPK pathways depending respectively on kinase *MgFus3, MgHog1* and *MgStl2* . These three signaling pathways are involved at different stages of the infection process (Cousin et al., 2006; Mehrabi et al., 2006; Mehrabi et al., 2006). To unravel the infection related mechanisms controlled by these signaling pathways, we are developing a combination of molecular approaches. Comparative transcriptomics will be performed using null mutants from these three genes (*MgFus3, MgHog1, MgStl2*) and wild type strains with genome wide DNA microarrays grown under conditions corresponding to either an active or an inactive pathway. Additional transcriptomic analyses will rely on conditional MAPK mutants to better control the activation/inactivation status of these pathways. Genes whose expression (induction, repression) require an active MAPK will be further studied using reverse genetics and biochemistry (phosphorylation status, protein-DNA interaction). Cousin et al. (2006), Molecular Plant Pathology 7(4): 269-278. Mehrabi et al. (2006), Molecular Plant-Microbe Interactions 19(4): 389-398. Mehrabi et al. (2006), Molecular Plant-Microbe Interactions 19(11): 1262-1269.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

142. A *Polyporus brumalis Laccase (pblac1)* was strongly induced by highly concentrated copper. Keiko Nakade, Yuko Nakagawa, Akira Yano and Yuichi Sakamoto Iwate Biotechnology Research Center, Iwate, Japan

Polyporus brumalis (*P. brumalis* IBRC05015) was isolated from north area of Japan, which had high ability to secrete proteins and high Laccase (Lac) productivity. In this study, to increase Lac productivity more, several inducers were searched in *P. brumalis* IBRC05015. High concentrated copper (0.1-0.5 mM) was the most effective inducer for Lac activity. PbLac1 was a Lac induced by copper in this strain. The *pblac1* mRNA accumulated by addition of copper. One copper responsible transcription factor Ace1 (Activation protein of cup1 Expression) binding element was found in *pblac1* promoter region. We cloned an Ace1 transcription factor homologue from *P. brumalis* IBRC05015 (PbAce1). From amino acid sequence of PbAce1, it was found that PbAce1 has well conserved Copper- fist DNA binding domain DBD (Copper-fist DBD ; ^{11}CysX2CysX8CysXHis^{25}), which is known as a both DNA and/or Cu binding in N-terminal region, and metallothionein-like cystain rich sequence in C-terminal region. PbAce1 complemented the function of *S. cerevisiae* Ace1 by heterologous expression of PbAce1 in delta ace1 strain. However, single or double amino acid change at the PbAce1 Copper-fist DBD Cys-11 and His-25 to Tyr lost the complement ability in *S. cerevisiae* delta *ace1* strain. These results showed that Copper-fist DBD is necessary for the function of PbAce1.

143. Light-dependent gene induction in *A. nidulans* requires release of the repressor LreA and binding of the activator FphA. Maren Hedtke, Julio Rodriguez-Romero and Reinhard Fischer Karlsruhe Institute of Technology, Dept. of Microbiology, Karlsruhe, Germany Maren.Hedtke@KIT.edu

Light serves as an important environmental signal to regulate development and metabolism in many fungi and has been studied to some detail in *N. crassa* and *A. nidulans*. *A. nidulans* develops mainly asexually in the light and sexually in the dark. The red-light sensor phytochrome (FphA) and the WC-1 homologue blue-light receptor LreA have been shown to mediate the light response in *A. nidulans* (1). There is evidence that both proteins form a light regulator complex (LRC). LreB (WC-2) and VeA are probably also components of this complex (2). Using ChIP and qRT PCR we show that HA-tagged FphA and LreA bind to the promoters of the *A. nidulans* homologues of *N. crassa con-10 (conJ)* and *ccg-1 (ccgA)*. *conJ* and *ccgA* are both induced during development but are also strongly upregulated after short exposure to light. Surprisingly we found LreA bound to the *conJ* and *ccgA* promoter only in the dark probably acting as a repressor. In contrast, FphA is recruited to the promoters after short illumination and seems to function as activator of transcription. These results suggest that the LRC is not a tight protein complex but rather transient and that light induction depends on derepression followed by induction through FphA. (1) Blumenstein A. et al., (2005) *Curr. Biol* 15(20):1833-8 (2) Purschwitz J., Müller S. & Fischer R., (2008) *Mol. Genet. Genomics* 18(4):255-9

144. The *fkhA* gene, encoding a forkhead protein, controls sexual development of *Aspergillus nidulans*. Dong- Soon Oh, Jong-Hwa Kim and Kap-Hoon Han. Department of Pharmaceutical Engineering, Woosuk University, Wanju, 565- 701. khhan@woosuk.ac.kr .

In a homothallic filamentous fungus *Aspergillus nidulans*, sexual development is largely affected by the genetic and environmental factors. To regulate the complex gene subsets involved in the sexual development accurately, tight regulations of transcription factors are required. The forkhead type transcription factors are the class of regulators that function in a broad spectrum of cellular and developmental processes in many species from yeast to human. Here, we identified the *fkhA* gene which encodes a putative forkhead transcription factor homologous to the yeast *FKH1* gene that is involved in sexual development. The *fkhA* deletion resulted in the complete loss of fruiting body formation under all conditions favoring sexual development, suggesting that the *fkhA* gene is required for sexual development in *A. nidulans*. Overexpression of *fkhA* resulted in enhanced formation of fruiting bodies under induction condition not only in the normal condition but also in the condition of presence of 0.6 M KCl which strongly inhibits sexual development. These results suggest that the *fkhA* gene is necessary and sufficient for regulating sexual development in *A. nidulans*. [This work was supported by the NRF grant 313-2008-2-C00804.]

145. Activation of Two Secondary Metabolite Gene Clusters in *A. nidulans* by Overexpression of Cross-Pathway Regulator ScpR. Funk, Alexander[1,5], Sebastian Bergmann[2,5], Peter Hortschansky[2], Kirstin Scherlach[3], Volker Schroeckh[2], Ekaterina Shelest[4], Uwe Horn[1], Christian Hertweck[3,5], and Axel A. Brakhage[2,5]. [1]Bio Pilot Plant, [2]Department of Molecular and Applied Microbiology, [3]Department of Biomolecular Chemistry, [4]Systems Biology/Bioinformatics Research Group Leibniz Institute for Natural Product Research and Infection Biology — Hans Knöll Institute (HKI), Jena, Germany [5]Friedrich Schiller University, Jena, Germany

Sequence data of the filamentous fungus and model organism *Aspergillus nidulans* provided insight into its biosynthetic potential. Yet the exact function and regulation of the majority of the speculated secondary metabolite pathways remains unknown, as most of these gene clusters are silent under standard laboratory conditions. Recent research focused on global mechanisms like regulation by LaeA as well as the role of locally acting transcription factors that are able to activate secondary metabolite pathways. The secondary metabolite cross-pathway regulator gene *scpR* is located in a cluster comprising two NRPS genes named *inpA* and *inpB* on chromosome II of *A. nidulans*. We show that overexpression of ScpR leads to transcriptional activation of the NRPS cluster on chromosome II as well as the *afo* cluster on chromosome VIII via additional activation of pathway-specific transcription factor AfoA. To the best of our knowledge, this is the first report of cross-pathway regulation of two different fungal biosynthesis gene clusters in *A. nidulans*. Financial support by the BMBF and TMBWK and the Jena School for Microbial Communication (JSMC) is gratefully acknowledged.

146. A forkhead protein FkhB affects conidiophore development in *Aspergillus nidulans* and *Aspergillus fumigatus*. Dong-Soon Oh, Jong-Hwa Kim and Kap-Hoon Han. Department of Pharmaceutical Engineering, Woosuk University, Wanju, 565-701, South Korea. khhan@woosuk.ac.kr .
Developmental process in a model filamentous fungus *Aspergillus nidulans* is controlled by the multiple regulatory systems including signal transduction pathways and transcription factors. We identified a forkhead type transcription factor which is conserved in *Aspergillus nidulans*, *Aspergillus fumigatus* and other relatives by using genome screening. The FkhB contains both of conserved forkhead domain (FH) and forkhead associated domain (FHA). To know the function of the FkhB proteins from both species, we deleted the *fkhB* genes in *A. nidulans* and *A. fumigatus*. The *fkhB* deletion in *A. nidulans* resulted in abnormal conidiophore formation under standard conditions. In *A. fumigatus*, *fkhB* knock-out mutant also produced severely reduced amount of conidiophores, suggesting that the *fkhB* gene plays an important role in sporulation both in *A. nidulans* and *A. fumigatus*. Furthermore, the phenotype is highly dependent in *veA* gene in *A. nidulans*. Taken together, the *fkhB* gene is a regulator of conidiation epistatic with the *veA* gene. [This work was supported by the NRF grant 313-2008-2-C00804.]

147. Withdrawn

148. Regulation of D-galactose metabolism; differences between yeast and filamentous fungi. Ulla Christensen[1], Birgit S. Gruben[2], Susan Madrid[1], Sara Hansen[1], Harm Mulder[1], Igor Nikolaev[1], Ronald P. de Vries[2,3]. [1]Danisco-Genencor, Leiden, The Netherlands; [2]Microbiology & Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, Utrecht, The Netherlands; [3]CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands

D-Galactose is present in hemicelluloses and pectin which are constituents of the plant cell wall. The synthesis of enzymes that are needed for the external release and the internal conversion of monosaccharides is tightly regulated, but can differ between fungal species. One of the best characterized regulators is the transcriptional activator Gal4 of *S. cerevisiae* which regulates genes from the metabolic Leloir pathway and genes encoding a galactose specific permease and an extracellular α- galactosidase. Gal4 is repressed by Gal80 in the absence of D-galactose, while Gal3 bound to ATP and D-galactose can relieve Gal4 from this repression. Galactose metabolism in filamentous fungi is regulated in a different manner, and involves two transcriptional activators, GalR and GalX, in *A. nidulans.* GalX activates the transcription of galR, which then regulates the transcription of genes from the metabolic Leloir pathway and the alternative galactose utilisation pathway. The interaction of the GalX and GalR regulators in *A. nidulans* and their control of the various genes of the two D- galactose utilization pathways will be compared to the regulatory mechanism of galactose metabolism in yeast. The presence of GalX and GalR throughout the fungal kingdom and the function of GalX in *A. niger* will be discussed.

149. *Aspergillus nidulans* regulatory subunit of protein kinase A, PkaR, is involved in sexual and asexual development as well as hyphal growth. Mi-Hye Park, Dong-Soon Oh, and Kap-Hoon Han. Department of Pharmaceutical Engineering, Woosuk University, Wanju, 565-701, South Korea. khhan@woosuk.ac.kr .

The cyclic AMP-dependent protein kinase A (PKA) is a well-known regulator of the growth, development and stress response in all eukaryotes. PKA consists of heterotetramer, made up of a dimmer of regulatory subunits and two catalytic subunits. It has been known that there are two PKA catalytic subunits, PkaA and PkaB, in a model filamentous fungus *Aspergillus nidulans*. PkaA and B play an important role in hyphal growth as well as asexual development process. Although many upstream signaling components including G protein coupled receptors, heterotrimeric G proteins, and their effectors have been studied, the function of regulatory subunit of PKA in *A. nidulans*, which is homologous of mammalian type II subunits and *BCY1* in *Saccharomyces cerevisiae* that is responsible for pseudohyphal growth and oxidative stress response, has remained to be elucidated. In this study, we constructed a deletion mutant of PKA regulatory subunit gene, *pkaR*, in *A. nidulans* and analyzed the effects. The *pkaR* deletion mutant showed restricted radial growth and diminished asexual development and defective in sexual development. Furthermore, delta *pkaR*; delta *pkaA* and delta *pkaR*; delta *pkaB* double mutants showed the additive phenotype in terms of hyphal growth and conidiation.

150. Characterization of *Mucor circinelloides* light-response mutants by high-throughput sequencing. Santiago Torres-Martínez, Eusebio Navarro and Victoriano Garre. Department of Genetics and Microbiology, Faculty of Biology, University of Murcia, Murcia 30071, Spain. storres@um.es

Light regulates developmental and physiological processes in a wide range of organisms, including fungi. Particularly, Zygomycete fungi have developed complex mechanisms to control the responses to light that await detailed characterization at molecular level. The basal fungus *Mucor circinelloides* is a good model for this purpose because its genome has been sequenced and several molecular tools are available for its manipulation. *Mucor*, like other Zygomycetes, has three *white collar-1* genes (*mcwc-1a*, *mcwc-1b* and *mcwc-1c*) that code for photoreceptor-like proteins. Analyses of knockout mutants suggest that each of these genes controls a specific response to light. Thus, *mcwc-1a* and *mcwc-1c* control phototropism and photocarotenogenesis, respectively. To identify new genes involved in regulation by light, a number of mutants showing either reduced carotene accumulation in the light or increased carotene accumulation have been isolated. Some of them present mutations on known structural and regulatory carotenogenic genes. High-throughput genome sequencing of others revealed the presence of non-conservative SNPs in 1 to 20 gene coding regions. Although some mutations map in genes of unknown function, others are in genes coding for proteins that may be involved in light transduction, such as a F-Box protein. Progress in the characterization of these genes in regulation by light will be shown.

151. Characterization of ZNF2 as a master regulator for hyphal morphogenesis and virulence in *Cryptococcus neoformans.* Linqi Wang and Xiaorong Lin£¬Texas A&M University, College Station, TX, xlin@mail.bio.tamu.edu

Dimorphism is a common feature that is usually associated with virulence potential in many dimorphic fungal pathogens. However, this association remains elusive in *Cryptococcus neoformans,* a major causative agent of fungal meningitis, in which filamentation is usually observed during mating. This is partially due to limited knowledge of filamentation-specific determinants in *C. neoformans*. We previously revealed that Znf2 is a terminal regulator for hyphal morphogenesis. Deletion of Znf2 completely abolishes filamentation and increases virulence in the animal model of cryptococcosis, suggesting that Znf2 plays a pivotal role in linking cryptococcal dimorphism and virulence. To further address the role of Znf2 in hyphal morphogenesis, we overexpressed Znf2 in the wild-type (JEC21|Á), as well as *mf|Á1,2,3|¤* and *mat2|¤* mutants in which the pheromone sensing pathway is blocked and self-filamentation is almost abolished. Overexpression of Znf2 in all backgrounds leads to extremely robust self-filamentation, indicating the role of Znf2 as a master regulator in cryptococcal self-filamentation. The effect of Znf2 overexpression on self- filamentation was found to be independent of serotype or mating type. Further dissection of roles of Znf2 in hyphal production and virulence is expected to provide not only the critical link for cryptococcal dimorphism and virulence but also a general mechanism underlying dimorphism and virulence among evolutionarily diverse fungal species.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

152. Throwing Light on Bikaverin Regulation in *Fusarium fujikuroi.* Philipp Wiemann and Bettina Tudzynski WWU Münster, IBBP, Schlossgarten 3, 48149 Münster, Germany

Fusarium fujikuroi is best known for its production of gibberellic acids (GAs). Besides GAs, *F. fujikuroi* may also synthesize other natural products such as carotenoids (CAR) and bikaverin (BIK). Recently, we were able to show that deletion of the global regulator of secondary metabolism and development, *velvet*, abrogates repressive cues such as alkaline pH and abundant nitrogen availability, resulting in BIK production under unfavored conditions. In this study, we extended our investigations on the influence of light and circadian rhythm. Unlike the early light- responsive CAR genes, the BIK genes are late light response genes. Furthermore, BIK production is an output of the circadian clock. Single and double deletions of genes coding for homologs of the *Neurospora crassa* GATA-type transcription factors white collar (WC)-1 and -2 result in 1) delay but not complete abrogation of CAR induction, 2) general upregulation of BIK genes but 3) WC-independent circadian BIK production. In contrast, the *velvet* deletion mutant has a derepressing effect on both pigments still exhibiting circadian rhythmicity. Our studies indicate that a putative white collar complex is linked to the *velvet*-like complex in *F. fujikuroi* thereby coordinating appropriate light response similar to the situation in *Aspergillus nidulans*. Furthermore, our data suggest that additional light-perceiving and rhythm-controlling factors must exist. The influence of phyto- and cryptochromes in this respect will be presented.

153. The nitrogen regulation network in *Fusarium fujikuroi*: sensing, signal transduction and cellular responses. Philipp Wiemann, Dominik Wagner, Caroline Michielse, Bettina Tudzynski Universität Münster, IBBP, Schlossgarten 3, D-48149 Münster, Germany

In *F. fujikuroi* the production of several secondary metabolites is regulated by nitrogen availability. Due to the strong impact of nitrogen on secondary metabolism, processes of nitrogen sensing, signal transduction and cellular responses on transcriptional and protein levels are focus of our interest. While gibberellins (GAs) are nitrogen-regulated in an AreA and AreB-dependent manner, the nitrogen-dependent expression of bikaverin (Bik) genes is regulated by a non-canonical AreA-independent mechanism. The contrasting mechanisms of nitrogen repression were confirmed by full derepression of Bik but not GA genes in the double AreA/MeaB and double AreA/AreB mutants. In contrast to GA and Bik genes, fusarin (Fus) gene expression depends on nitrogen sufficiency. The ammonium permease MepB is involved in sensing extracellular nitrogen, and *mepB* mutants revealed a deregulated GA, Bik and Fus gene expression. On the other hand, glutamine synthetase (GS) plays an important role in sensing the intracellular nitrogen status. In additon, GS seems to be an essential component of a regulatory circuit. Its deletion abolishes the expression of both nitrogen-repressed (GA, Bik) and nitrogen-induced (Fus) secondary metabolism genes. Interestingly, GSI and GSII from *Streptomyces* restored both the enzymatic activity of GS and secondary metabolism suggesting conserved regulation mechanisms between eukaryotic and prokaryotic GS proteins. We propose a model for the nitrogen regulation network with the main players depicted.

154. Investigating the Regulation of *gliA* Expression in *Aspergillus fumigatus*. Taylor Schoberle, Jennifer Herold, Gregory S. May. Department of Pathology and Laboratory Medicine, UT M. D. Anderson Cancer Center, Houston, TX.

The secondary metabolite gliotoxin has already been shown to be essential for full virulence in certain animal models. According to microarray studies, *gliA*, the gliotoxin transporter, is induced over 32-fold in the presence of Neutrophils. The gliotoxin biosynthetic cluster is co-regulated, so an increase in *gliA* expression is likely representative of the entire gene cluster. Unfortunately, there is little information pertaining to specific proteins that regulate the expression of the gliotoxin biosynthetic cluster. GliZ, a Zn_2Cys_6 transcription factor located within the cluster, and LaeA, a methyltransferase, are two known regulators of gliotoxin production, although LaeA also regulates the expression of a variety of other secondary metabolites within *A. fumigatus*. Using a genetic screen, our lab has discovered two novel proteins which significantly induce the expression of *gliA* when over-expressed. One of the proteins, *gipA*, which induces *gliA* over 400-fold, is a C_2H_2 transcription factor. Interestingly, over-expression of *gliZ* in the same conditions only induces *gliA* 10-fold. In non-inducing conditions, where the production of gliotoxin is minimal, two independent strains over-expressing *gipA* produce significantly higher levels of gliotoxin than the control strain. Furthermore, RNA transcript levels of five separate genes within the gliotoxin cluster are significantly up-regulated compared to an empty vector control strain. Another gene, discovered from the screen, significantly induces *gliA* expression (over 100-fold) and encodes a Sensor Histidine Kinase/Response Regulator. I hypothesize that *gliA* is regulated by a signal transduction pathway involving a novel transcription factor, *gipA*, and a novel Sensor Histidine Kinase/Response Regulator, *gipB*.

155. *Aspergillus fumigatus velvet* regulators. Hee-Soo Park, Nak-Jung Kwom, and Jae-Hyuk Yu* Department of Bacteriology, University of Wisconsin, Madison WI 53706 USA

Fungal development and secondary metabolism are intimately associated via activities of new class of the novel regulators, called the *velvet* proteins (VeA, VelB VelC and VosA) that are highly conserved in filamentous fungi. Here we investigated the roles of the *velvet* genes in the opportunistic fungal pathogen *Aspergillus fumigatus*. To study the function of the *Afuvelvet* genes, we generated individual deletion mutants in *A. fumigatus*. The absence of *AfuveA* and *AfuvelB* resulted in the formation of conidiophores and increased *AfubrlA* mRNA accumulation in liquid submerged culture where WT strains do not develop. During the progression of asexual development, the deletion of *AfuveA* or *AfuvelB* caused highly increased *AfubrlA* and *AfuabaA* mRNA accumulation. Levels of the *AfuvosA* and *AfuvelB* transcripts are high in conidia and during the late phase of conidiation, suggesting that *AfuvelB* and *AfuvosA* may play important roles in sporogenesis. In fact, the deletion of *AfuvosA* or *AfuvelB* caused reduced conidial trehalose amounts, spore viability and conidial tolerance to oxidative stress. These results suggest that the *Afuvelvet* genes differentially function in controlling trehalose biogenesis and spore maturation, and negative feed-back regulation of asexual development in *A. fumigatus*, and indicate the conserved functions of the *velvet* genes in aspergilli.

156. The chromodomain protein CDP-2 modulates DNA methylation in *Neurospora crassa*. Shinji Honda, Zachary A. Lewis, Tamir K. Khalafallah, Eun Y. Yu, Michael Freitag, Wolfgang Fischle, and Eric U. Selker

DNA methylation is involved in gene silencing and genome integrity in mammals, plants and some fungi. Abnormal DNA methylation is often associated with various defects in these organisms. In Neurospora, DNA methylation is directed by H3K9me3 deposited by the H3K9 methyltransferase DIM-5. HP1 acts as a bridging protein between H3K9me3 and the DNA methyltransferase DIM-2. We report identification of CDP-2, a chromodomain protein that binds to methylated H3K9. CDP-2 is colocalized with HP1 to heterochromatic foci in an H3K9me3-dependent manner. We found that CDP-2 stability depends on HP1 and that CDP-2 interacts with the chromoshadow domain of HP1 through a PXVXL-like motif near the N- terminus. Mutants lacking CDP-2 show region-specific hypomethylation, caused by mislocalization of DIM-5, and hypermethylation of centromeric heterochromatin, which results from enhanced association of DIM-2. CDP-2 is also required for spreading of DNA methylation in *dmm* mutants. CDP-2-mediated heterochromatin formation is essential for normal growth when DNA methylation is absent. We conclude that CDP-2 modulates self-reinforcing loops between H3K9me3, HP1 and DNA methylation to maintain silent chromatin.

157. Evidence that HxkC, an *Aspergillus nidulans* mitochondrial hexokinase-like protein, is anti-apoptotic Margaret E. Katz[1], Rebecca Buckland[1], and Matthias Brock[2], [1]Molecular and Cellular Biology, University of New England, Armidale, NSW 2351 Australia, mkatz@une.edu.au [2]Microbiell Biochemistry, Hans-Knoell-Institut, Beutenbergstr. 11a, Jena 07745, Germany, Matthias.Brock@hki-jena.de

Binding of hexokinase II to mitochondria inhibits Bax-induced cytochrome *c* release from mitochondria and apoptosis in mammalian cells (Pastorino et al, 2002). HxkC, which plays a role in the response to nutrient stress, is the first fungal hexokinase shown to be associated with mitochondria (Bernardo et al. 2007). In a strain lacking functional HxkC, cleavage of DNA into oligonucleosomal fragments, a hallmark of mammalian apoptosis, occurs even in the absence of nutrient stress. This suggests that, as in plants, a fungal mitochondrial hexokinase inhibits programmed cell death even though Bax, a member of the Bcl-2 family, is not present. The *hxkC delta* null mutant shows increased susceptibility to oxidative stress but increased resistance to rapamycin-induced-inhibition of conidiation. Higher levels of intracellular protease activity, which could be the result of autophagy, are detected in the *hxkC delta* mutant. To determine whether HxkC plays a role in autophagy, we have generated mutants that lack both HxkC and AtgA, Although no loss of hexokinase activity was detected in the *hxkCdelta* mutant, purification of HxkC has revealed that the protein possesses low levels of ATPase and glucose-phosphorylating activity. Pastorino J.G., Shulga N., Hoek J.B. (2002) Mitochondrial binding of hexokinase II inhibits Bax-induced cytochrome *c* release and apoptosis. *J. Biol. Chem.* **277:** 7610-7618. Bernardo S.M.H., Gray K.-A., Todd R.B., Cheetham B.F., Katz M.E. (2007) Characterization of regulatory non-catalytic hexokinases in *Aspergillus nidulans. Mol. Genet. Genomics* **277:** 519-532.

158. The NsdD, a GATA type transcription factor that controls sexual development of *Aspergillus nidulans*, localizes in nuclei during late vegetative growth. Jae-Sin Park, Lee-Han Kim, Jung-Su So and Dong-Min Han Division of Life Science, Wonkwang University

The *nsdD* gene encodes a GATA-type transcription factor carrying the type IVb zinc finger DNA binding domain at its C-terminus, which controls sexual development positively in *A. nidulans*. An NLS is found in near zinc finger domain suggesting that the NsdD be localized in nucleus. The monomeric red fluorescent protein (mRFP) ORF was fused downstream of the *nsdD ORF* and forcedly expressed under alc promoter. Low level of the fluoroscent signal was detected in conidia and germlings inconsistently. The fluorescence was concentrated in nuclei in the hyphe grown for 16h indicating that the polypeptides are transported into nuclei at late vegetative growth stage. The fluorescent sinals were not found in all asexual sporulation structures or Hulle cells. However, asci and ascospores are filled with the polypeptides implicating the additional function of NsdD in late sexual sporulation. The nuclear localization of NsdD after 16h growth coincides with the time of repression of a positive regulator of asexual development, NrsA, by NsdD and VeA, suggesting that NsdD enters nucleus at late stage of vegetative growth and regulates the expression of *nrsA* negatively leading the hyphae to undergo sexual development.

159. Aspergillus fumigatus flbB encodes two basic leucine zipper domain (bZIP) proteins required for proper asexual development and gliotoxin production. Peng Xiao[1,2], Kwang-Soo Shin[3], Tianhong Wang[1],and Jae-Hyuk Yu[2*1]State Key Laboratory of Microbial Technology, Shandong University, Jinan, P. R. China. [2]Departments of Bacteriology and Genetics, University of Wisconsin, Madison, Wisconsin. [3]Department of Microbiology and Biotechnology, Daejeon University, Daejeon, Republic of Korea.

The opportunistic human pathogen *Aspergillus fumigatus* reproduces asexually by forming a massive number of mitospores called conidia. In this study, we characterize the upstream developmental regulator *A. fumigatus flbB* (*AfuflbB*). Northern blotting and cDNA analyses reveal that *AfuflbB* produces two transcripts predicted to encode two basic leucine zipper domain (bZIP) polypeptides, *Afu*FlbBbeta (420 amino acids [aa]) and *Afu*FlbBalpha (390 aa). The deletion of *AfuflbB* results in delayed/reduced sporulation, precocious cell death, the lack of conidiophore development in liquid submerged culture, altered expression of *AfubrlA* and *AfuabaA*, and blocked production of gliotoxin. While introduction of the wild-type (WT) *AfuflbB* allele fully complemented these defects, disruption of the ATG start codon for either one of the *Afu*FlbB polypeptides leads to a partial complementation, indicating the need of both polypeptides for WT levels of asexual development and gliotoxin biogenesis. Consistent with this, *Aspergillus nidulans flbB*$^{+}$ encoding one polypeptide (426 aa) partially complements the *AfuflbB* null mutation. The presence of 0.6 M KCl in liquid submerged culture suppresses the defects caused by the lack of one, but not both, of the *Afu*FlbB polypeptides, suggesting a genetic prerequisite for *Afu*FlbB in *A. fumigatus* development. Finally, Northern blot analyses reveal that both *AfuflbB* and *AfuflbE* are necessary for expression of *AfuflbD*, suggesting that FlbD functions downstream of FlbB/FlbE in aspergilli.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

160. A split luciferase complementation assay for studying *in vivo* protein-protein interactions in the plant pathogenic ascomycete *Gibberella zeae.* Bo Reum Sung, Seong mi Jo, Eun Ji Cho, Hee-Kyoung Kim, and Sung-Hwan Yun. Department of Medical Biotechnology, Soonchunhyang University, Asan 336-745, Korea

Various types of protein-protein interactions play important roles in controlling fundamental cellular processes. To date, several techniques (e.g. yeast two hybrid, FRET, BiFC) have been used for the detection of protein-protein interactions in living cells. As an alternative method, split luciferase complementation, which provides an amplified signal for detecting weak protein interactions, has been developed in animal and plant cells. Here we examined if the split luciferase assay could be employed in *Gibberella zeae*. The nucleotide sequences of two strongly interacting proteins (a F-box protein, FBP1 and its interacting partner SKP1), which were under control of the cryparin promoter from *Cryphonectria parasitica*, were translationally fused to the N- and C-terminal fragments of the luciferase from *Renilla reniformis*. Each fusion product was cloned into a fungal transforming vector carrying gene for the resistance to either hygromycin B or geneticin, and transformed into a wild-type *G. zeae* strain (Z03643). We detected the protein interaction by a high luminescence intensity-to-background ratio detected only in the cell-free extracts from the fungal transformants expressing both fusion constructs. This study demonstrates that the split luciferase complementation would be a sensitive and efficient way to study *in vivo* protein-protein interaction in *G. zeae*.

161. Quantitative analysis of transcripts that encode lignocellulose-active enzymes expressed by *Phanerochaete carnosa* at progressive stages of wood decay. Jacqueline MacDonald[1] and Emma Master[1,2] [1]Department of Chemical Engineering & Applied Chemistry, and [2]Department of Cell & Systems Biology, University of Toronto, Toronto, Canada

Wood-decaying white-rot fungi secrete enzymes that can degrade all of the main components of lignocellulose and so are a valuable source of enzymes used in the production of renewable chemicals and liquid fuel from wood. While most white- rot have been isolated primarily from hardwoods (angiosperms), *Phanerochaete carnosa* has been isolated almost exclusively from softwoods (gymnosperms). It is anticipated that by elucidating the enzyme activities that facilitate softwood decay by *P. carnosa*, new enzyme formulations will be identified that result in more efficient utilization of this resource. Previous transcriptome analysis of *P. carnosa* identified transcripts that are enriched during growth on wood substrates. To gain a better understanding of wood decay by this fungus, we used quantitative (q) RT-PCR to quantify transcripts encoding manganese peroxidases (MnP), lignin peroxidases, mannanase, xylanase, acetyl xylan esterase, glucuronoyl esterase, and cellobiohydrolase at five time points during cultivation on balsam fir, lodgepole pine, white spruce or sugar maple. Transcript profiles are consistent with a concerted response to wood species and a sequential decay strategy where lignin is decayed early on. Compared to reports of the model hardwood-degrading *Phanerochaete chrysosporium*, *P. carnosa* produces a greater proportion of transcripts encoding proteins involved in lignin decay, particularly MnP. We also evaluated three internal standards for qPCR, and found transcripts encoding chitin synthase to be more consistently expressed than actin or GAPDH.

162. Small RNA mediated meiotic silencing of a transposable element in *Neurospora crassa*. Yizhou Wang, Kristina Smith, Michael Freitag, and Jason E. Stajich, University of California, Riverside, CA ywang039@student.ucr.edu

Meiotic silencing of unpaired DNA is a genome defense mechanism that occurs in *Neurospora crassa* (Shiu et al, 2001; Shiu et al, 2002). The mechanism of silencing is hypothesized to be through the production of small RNAs specific to the unpaired regions and likely works to prevent the spread of invading elements such as transposable elements (TE). Through previous work we identified a novel TE that is ~10kb in length in 1-2 copies in the reference genome of N. crassa OR74A but is missing in most other strains. The TE is a member of a new superfamily of DNA TEs related to Mutator Like Elements (MULEs). A cross of strains where only one parent contains the TE should induce meiotic silencing and smallRNA sequencing should reveal the presence of the small RNAs specific to the unpaired region. Mycelia and protoperithecial tissue from OR74A and mycelia and perithecia were collected from 2 and 4 days after fertilization with a strain lacking the TE (D60; FGSC#8820). Total RNA was extracted and subjected to small RNA Illumina sequencing. The most abundance of smallRNA mapping to the TE was found in the 4 days post fertilization while few small RNAs were produced in the protoperithecia or found in other available small RNA libraries from vegetative tissues. Northern blots of the small RNAs from the TE region verified the sequencing observations. These results provide strong support for endogenous silencing role of meitoic silencing an intact transposable element and identify small RNAs produced specifically from an unpaired region.

163. Analysis of genome-wide binding by a transcription factor involved in fatty acid metabolism, FAR-1. Erin L. Bredeweg[1], Fei Yang[2], Kristina M. Smith[1], Rigzin Dekhang[2], Jillian M. Emerson[3], Jay C. Dunlap[3], Deborah Bell-Pedersen[2], Matthew S. Sachs[2] and Michael Freitag[1]. [1]Program for Molecular and Cellular Biology, Department of Biochemistry and Biophysics, and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331; [2]Department of Biology and Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX; [3]Department of Genetics, Dartmouth Medical School, Hanover, NH.

In a previous study we analyzed genome-wide binding of the WHITE COLLAR-2 (WC-2) transcription factor (TF) after a short light pulse. We found that ~20 TF genes showed enrichment of WC-2 in their promoter regions. Curiously, a small subset of TF genes showed WC-2 binding in the absence of mRNA light induction. One such gene, *far-1* (NCU08000), also known as the gene for "cutinase transcription factor 1-alpha" (CTF1α) in *Nectria haematococca* or the "fatty acid regulator" (FarA) in *Aspergillus nidulans*, showed small but significant enrichment of WC-2. We tagged *far-1* at the C-terminus with GFP and carried out ChIP-sequencing to find potential binding sites. We found that most regions with FAR-1 enrichment contained a previously identified 5'-CCGAGG-3' consensus sequence. As expected from studies in other organisms, FAR-1 bound to promoters of genes involved in beta- oxidation, peroxisomal and mitochondrial processes. Nevertheless, we found additional binding sites and some of these were occupied in a light-dependent manner. In addition, WC-2 and FAR-1 appeared to bind to subset of promoters in a coordinated fashion.

164. Identification and characterization of aquaporins and aquaglyceroporins in *Aspergillus fumigatus* and *Aspergillus nidulans*. Dong-Soon Oh, Hanyan Lu, Haojun Lian, Xiao Hu and Kap-Hoon Han. Department of Pharmaceutical Engineering, Woosuk University, Wanju, 565-701, South Korea. khhan@woosuk.ac.kr .

Aquaporin is a water channel protein, which is related in Major Intrinsic Protein (MIP), found in almost all organisms from bacteria to human. To date, more than 200 members of this family were identified. There are two major categories of MIP channels, aquaporins and glycerol facilitators, which facilitate the diffusion across biological membranes of water or glycerol and other uncharged compounds, respectively. The full genome sequencing of various fungal species revealed 3 to 5 aquaporins in their genome. However, no functional characteristics were studied so far in Aspergillus sp. In *Aspergillus nidulans*, one orthodox aquaporin (*aqpA*) and four aquaglyceroporins (*aqpB~E*) were found and one orthodox aquaporin and two aquaglyceroporins were found in *Aspergillus fumigatus*. In *A. nidulans*, knock-out of each aquaporin or aquaplyceroporin didn't show obvious phenotypic change in osmotic stress, indicating the function of the genes are redundant. However, oxidative stress and antifungal susceptibility has been changed in some mutants. Furthermore, *A. fumigatus* aquaporins disruption resulted in sensitive in osmotic stress as well as oxidative stress, suggesting that the function of aquaporins in *A. fumigatus* play roles in regulation both of osmotic stress and oxidative stress without redundant manner [This work was supported by NRF Korea (2009- 0072920)].

165. Osmoregulation in fungi isolated from solar salterns: HOG signal transduction pathway in extremely salt tolerant fungus *Hortaea werneckii* and halophillic fungus *Wallemia ichthyophaga* [a] Ana Plemenitas , [a] Metka Lenassi, [a] Martin Fettich, [a] Tilen Konte and [b] Nina Gunde- Cimerman [a] Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia [b] Biology Department, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia E-mail:ana.plemenitas@mf.uni-lj.si

Solar salterns and other similar hypersaline environments are extreme habitat that prevent growth of most organisms except those, well adapted to extremely high salt concentration. Discovery of the extremely salt tolerant fungus *Hortaea werneckii* as the dominant fungal species in hypersaline waters enabled the introduction of a new model organism to study the mechanisms of salt tolerance in eukaryotes and a promising source of transgenes for osmotolerance improvement of industrially important yeasts, as well as in crops. While *H.werneckii* is unique in its adaptability to fluctuations in salt concentration and grow without NaCl as well as in the presence of up to 5M NaCl, we have recently introduced another model organism, fungus *Wallemia ichthyophaga* which is a true halophile in a sense that it requires the presence of at least 10% of NaCl for the growth and grows also in the presence of up to 5M NaCl. Sensing changes in sodium concentrations in the environment and responding to them is vital for cell survival. We have identified in *H.werneckii* and in *W.ichthyophaga* the genes that code for the components of pathway homologous to the HOG signal transduction pathway in *Saccharomyces cerevisiae* : sensor proteins Sho1 and Sln together with the homologues of MAP kinases Ste11, Pbs2 and Hog1. Functional complementation of the identified genes in *S.cerevisiae* mutant strain revealed some of their functions. Analysis of the identified proteins demonstrated important structural differences between the components of HOG pathway in fungi, isolated from solar saltern and *S.cerevisiae* as well as between extremely salt tolerant *H. werneckii* and halophillic *W. ichthyophaga*.

166. Transcriptional repressor RCO-1 controls the *ccg-13* and *ccg-14* genes under the OS-2 MAP kinase in *Neurospora crassa*. Kazuhiro Yamashita, Masakazu Takahashi, Masayuki Kamei, Akihiko Ichiishi, Makoto Fujimura. Faculty of Life Sciences, Toyo University, Itakura, Gunma, Japan.

We previously reported that ATF-1 acts as one of the transcriptional factors downstream of the OS-2 MAP kinase and regulates a large number of genes including some of clock-controlled genes. The expression of *ccg-1, ccg- 9, ccg-13, ccg-14* and *bli-3* is stimulated by activation of OS-2 MAP kinase. Among them, *ccg- 13* and *ccg-14* are upregulated in OS-2-dependent but ATF-1-independent manner, suggesting the existence of another transcription factor regulated by OS-2. Based on the deletion assay of the *ccg-13* promoter, we found the repressive element between -1200 bp and -1000 bp upstream of ATG. We purified the proteins that bind the repressive element of *ccg-13* using streptavidin magnetic beads and identified its protein as RCO-1 (regulator of conidiation-1) by MALDI-TOF mass spectrometry. Constitutive expression of the *ccg-13* and *ccg-14* was observed in the *rco-1* disruptant indicating that RCO-1 is a negative transcriptional factor regulated by the OS-2 MAP kinase. It is known that *rco-1* mutant have elevated expression of several of the conidiation-specific genes and null alleles fail to produce mature conidiophores and release free conidia. ATF-1 are known to be involved in conidial viability. These findings indicate that OS-2 MAP kinase plays an important role not only for stress adaptation but also asexual differentiation through the two transcription factors ATF-1 and RCO-1 in *Neurospora crassa*.

167. Sterol-Regulatory Element Binding Protein, SrbA is required for hypoxic adaptation and ergosterol synthesis in *Aspergillus nidulans*. Mee-Hyang Jeon, Sun-Ki Koh, Jun-Yong Kwak, Chinbayar Bat-Ochir, and Suhn-Kee Chae Department of Biochemistry and Fungal Pathogenesis Center, Paichai University, Daejeon 302-735, Korea

Sterol Regulatory Element Binding Proteins (SREBPs), a family of membrane-tethered transcription factors regulate sterol synthesis in mammals and hypoxic gene expressions in *S. pombe* and *A. fumigatus*. In this study, an SREBP homolog *srbA* in *A. nidulans* was cloned and analyzed. Null mutants of *srbA* exhibited no growth in hypoxia and high sensitivity to itraconazole, an inhibitor of lanosterol 14-alpha demethylase (LAD). The mutant phenotype of *srbA* null mutants was rescued by over-expression of SrbA and the SrbA N-terminus but not with the C-terminus. Two genes of *erg11A* and *erg11B* encode LADs in *A. nidulans*. Δ*erg11A* showed high itraconazole sensitivity and no growth in hypoxia, while Δ*erg11B* exhibited no difference to wild type. Double mutants of Δ*erg11A* and Δ*erg11B* were lethal. Hypoxia caused enhanced expression of *erg11A* but not *erg11B*. In Δ*srbA*, expression of *erg11A* was highly decreased. SrbA also affected expressions of other genes in the ergosterol synthesis pathway. [Supported by NRF of Korea]

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

168. Characterization of the *insA* Gene Encoding INSIG a Component of the SREBP pathway in *Aspergillus nidulans.* Chinbayar Bat-Ochir, Sun-Ki Koh, Mee-Hyang Jeon, and Suhn-Kee Chae Department of Biochemistry and Fungal Pathogenesis Center, Paichai university, Daejeon 302-735, Korea

Cholesterol synthesis in mammals is controlled by Sterol Regulatory Element Binding Proteins (SREBPs). Ergosterol synthesis of *S. pombe* is also regulated by a mammalian SREBP pathway like regulatory system. Moreover this system is not only responsible for ergosterol synthesis but also necessary for hypoxic adaptation. The homologs of the SREBP and INSIG were also found in *Aspergillus nidulans* and named *srbA* and *insA*, respectively. Our previous results showed that *srbA* was required for both ergosterol synthesis and growth in hypoxia. In the presence of itraconazole and in hypoxia, expression of *insA* was reduced, while level of *srbA* transcripts was enhanced. Forced overexpression of *insA* caused reduction of *srbA* expression in hypoxia and resulted in high sensitivity to itraconazole. Co-overexpression of *srbA* rescued the itraconazole-sensitive phenotype in *insA* overexpressed strains. In *insA* null mutants the level of *srbA* transcripts in normoxia was enhanced similar to that in hypoxia. These results implied that InsA played a negative role on the expression of *srbA*. [Supported by NRF of Korea]

169. The velvet-like complex from chrysogenum: A regulatory network of five subunits controls secondary metabolism and morphogenesis. Kück U[1], Hoff B[1], Kamerewerd J[1], Kopke K[1], Wolfers S[1], Katschorowski A[1], Milbredt S[1], Koutsantas K[1], Kluge J[1], Zadra I[2], Kürnsteiner H[2] [1]Christian Doppler Laboratory for Fungal Biotechnology, Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Universitätsstraße 150, 44780 Bochum; [2]Sandoz GmbH, 6250 Kundl, Austria

The global regulator velvet together with laeA is a core component of the velvet complex from Aspergillus nidulans. We have characterized a velvet-like complex from the penicillin producer Penicillium chrysogenum, which contains at least five different subunits. Included is PcvelA P. chrysogenum, which is an inhibitor of light-dependent conidiation and affects the biosynthesis of the beta-lactam antibiotic penicillin*. We will present an extensive analysis of subunits PcvelB, PcvelC and PcvosA of the velvet-like complex, including data from array hybridization, high performance liquid chromatography, quantification of penicillin titers, microscopic investigations and mass spectrometry. We provide evidence that all subunits of this complex have conserved as well as novel roles in secondary metabolism and morphogenesis in P. chrysogenum. These results confirm and extend the current picture of regular networks controlling both, fungal secondary metabolism and morphogenesis. *Hoff et al. EUKARYOTIC CELL 9: 1236–1250 (2010)

170. Using codon-improved GFP for imaging gene expression and differentiation in germinating spores of *Botrytis cinerea.* M.Leroch, T.Coenen, D.Koppenhöfer, D.Mernke, M.Hahn Dept.of Biology, University of Kaiserslautern, P.O. box 3049, 67653 Kaiserslautern, Germany, E-mail: hahn@rhrk.uni- kl.de

Spore germination is a fundamental event in fungal life, representing initiation of growth from a dormant state. In plant pathogens, germination immediately precedes host penetration and is of crucial importance for successful infection. We have performed transcriptome studies of early differentiation of *Botrytis cinerea* conidia. Massive changes in gene expression were observed already after 1 hour, before germ tube emergence. Genes upregulated during germination and appressorium formation (1-4 h.p.i.), were found to be enriched in genes encoding secreted proteins, indicating a strong secretory activity during the early stages of development. In a mutant lacking BMP1 MAP kinase, which is essential for host penetration and infection, upregulation of many secretory genes was not observed. We have developed a codon- improved, intron-containing version of *egfp*, yielding ten-fold higher GFP fluorescence compared to *egfp* in *B. cinerea*. Promoter-GFP reporter strains confirmed germination-specific expression for several genes and allowed live imaging studies of the infection process. In particular, expression of several cutinase genes on the host surface was dependent on contact-dependent germination and the presence of hydrophobic substrates (cutin monomers or waxes) from the plant surface. These data demonstrate an early molecular communication between pathogen and host which starts during or even before germ tube emergence.

171. The *Neurospora crassa* PacC transcription factor binds to *gsn* promoter and modulates the gene expression under extracellular pH changes. Fernanda Barbosa Cupertino, Fernanda Zanolli Freitas, Maria Célia Bertolini, Instituto de Química, UNESP, Departamento de Bioquímica e Tecnologia Química, Araraquara, SP, Brazil, mcbertol@iq.unesp.br

In *N. crassa*, the *gsn* gene encoding glycogen synthase is regulated under different environmental conditions. Previous results from our laboratory have demonstrated that the glycogen accumulation and the *gsn* gene expression is modulated upon ambient pH changes. The glycogen accumulation was decreased and the *gsn* gene was down-regulated under alkaline pH characterizing *gsn* as an acid-specific gene. In *Aspergillus nidulans* gene expression regulation under alkaline growth conditions is mediated by the PacC transcription factor. The *gsn* promoter contains a *cis* PacC DNA element leading us to investigate whether the NcPacC would be able to recognize and bind to this regulatory element. The recombinant protein was produced in *E. coli* fused to His tag, purified by affinity chromatography and its ability to bind to a DNA fragment (146 bp) containing the PacC motif was analyzed by gel shift experiments. A DNA-protein complex of high molecular weight was observed, and the specificity was confirmed by using the same DNA fragment as specific competitor. The complex specificity was probed by preparing a double-strand DNA oligonucleotide (27 bp) containing the core sequence of the PacC motif and by constructing a mutant DNA fragment by site-directed mutagenesis. The results indicate that under alkaline pH the NcPacC protein binds to the *gsn* promoter, leading to its transcription down-regulation, and a decrease in the glycogen accumulation. Financial support: FAPESP, and CNPq

172. Calcineurin and its Regulator Calcipressin - The Calcium Signaling Network in *Botrytis cinerea.* Karin Harren, Julia Schumacher and Bettina Tudzynski IBBP, WWU Münster, Schlossgarten 3, 48149 Münster, Germany, karin.harren@uni-muenster.de

The Ca^{2+}/calmodulin-dependent phosphatase calcineurin (CN) is a conserved protein that plays a critical role in Ca^{2+} signaling and stress response. Inhibitor studies with Cyclosporine A showed that the expression of several genes, e.g. those involved in phytotoxin biosynthesis, is regulated by CN and the CN-responsive transcription factor CRZ1 in *B. cinerea*. CN activity itself is regulated by a class of conserved proteins termed Calcipressins. Deletion mutants of *bccnA* encoding the catalytic subunit of CN are strongly impaired in growth and development and form small, compact colonies. In comparison, deletion of the only calcipressin homologous gene in *B. cinerea*, *bcrcn1*, affects vegetative growth in a similar way as the *bccnA* deletion mutant. Remarkably, improved growth of both mutants is observed on media containing high sugar concentrations or sodium chloride. While *bccnA* deletion mutants are totally apathogen, *bcrcn1* deletion mutants show significantly reduced virulence on living bean plants. Based on Northern studies with CN-dependent genes as probes we suggest that BcRCN1 functions as an activator of BcCNA. Current investigations focus on motifs which modulate the regulating effect of BcRCN1 on BcCNA. Furthermore, analyses of *bcrcn1* deletion mutants expressing a CRZ1-GFP fusion protein or the intracellular Ca^{2+} reporter Aequorin, respectively, will bring new insights into the complex Ca^{2+} signaling network involved in development and virulence of *B. cinerea*.

173. RNA silencing and Mycoviruses in *Botrytis cinerea*. Seuseu Tauati [1], Mike Pearson [2], Mathias Choquer [3], Gary Foster [1] Andy Bailey [1] [1]School of Biological Sciences, University of Bristol, UK. [2] School of Biological Sciences, Auckland University, NZ. [3]Bayer Crop Science, Lyon France.

Botrytis cinerea is the cause of the gray mould disease, a common infection of many fruit and vegetables. It is controlled by the use of both cultural and chemical methods, but it would be interesting to see if mycoviruses were a feasible method for reducing fungal virulence and thus controlling disease. Before this is a realistic possibility, more has to be understood of the RNA silencing mechanism which may act as a cellular defence system against viral infection. Analysis of the genome data for B. cinerea identified two dicer genes (Dcr1 and Dcr2). Targeted gene disruption was successfully used to create two independent B. cinerea Dcr2 mutants in a KU70 background. To determine whether RNA silencing was still active, the mutants were transformed with an argininosuccinate synthetase (bcass) silencing cassette. Many of these transformants displayed partial auxotrophy showing that silencing was still effective in a Dcr2 mutant. This suggests that the silencing machinery in B. cinerea is more like the situation in N. crassa where either dicer was sufficient to cause silencing. The sequenced single stranded RNA mycovirus Botrytis virus F (BVF) has transfected into wild-type and Dcr2-disrupted B. cinerea lines and results will be presented showing the impact of this virus on expression of various candidate genes.

174. Asexual development is regulated by histone H3 acetylation in Aspergillus nidulans. David Cánovas*, Yazmid Reyes-Domínguez#, Ana T. Marcos*, Ulrich Güldener‡, and Joseph Strauss# *Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Spain, davidc@us.es; #Fungal Genetics and Genomics Unit, AIT-Austrian Institute of Technology and BOKU University, Vienna, Austria. ‡Institute of Bioinformatics and Systems Biology, Helmholtz Center Munich, Germany

The ascomycete Aspergillus nidulans is a model organism to study fungal development. The expression of the brlA gene triggers the formation of the developmental structures, the conidiophores. The expression of brlA is regulated by a number of upstream regulators, including FluG, FlbA-E. In addition to these regulators, we have found that the product of gcnE is necessary for the expression of the brlA gene. GcnE is a homolog of yeast GCN5p, the catalytic subunit of the conserved SAGA/ADA complexes responsible for the majority of lysine acetylation in histone H3 (H3ac) and subsequent transcription-related chromatin remodelling. In A. nidulans, deletion of gcnE results in a severe defect of asexual development. In this study, we compared wild type and the gcnEΔ mutant by transcriptome analysis and chromatin modification assays. Microarray analysis revealed major effects on the expression of genes involved in primary and secondary metabolism as well as in development. Consistently, the expression of brlA is dramatically reduced and some of the upstream regulators are deregulated. Chromatin immunoprecipitation assays revealed an altered pattern of H3ac in promoters of the conidiation regulators, suggesting that H3 acetylation carried out by GcnE is required for the accurate regulation of conidiation.

175. The cell wall integrity signalling in *Aspergillus fumigatus* regulates secondary metabolites production and iron homeostasis. Valiante, V., Jain, R., Heinekamp, T., Remme, N., Haas, H., Brakhage, A.A.

Responding to external signals and adaptation to changes in the milieu is indispensable for the viability of all living organisms. *Aspergillus fumigatus*, a sophisticated saprophytic fungus is able to grow and proliferate in a variety of environments. Fungal pathogens such as *A. fumigatus* employ signal transduction cascades such as Mitogen Activated Protein Kinase (MAPK) pathways to sense, transduce and regulate different developmental processes of the fungal cell in response to extracellular cues. The genome of *A. fumigatus* harbours four MAPK genes. One of them, named as MpkA, has been shown to be a key player of the cell wall integrity (CWI) pathway of *A. fumigatus*. We made a comprehensive study of the role of the CWI pathway in *A. fumigatus*. We found that MpkA regulated CWI signalling is involved in regulation of plethora of genes ranging from those involved in cell wall repair and synthesis, defence against oxidative stress, pigment and toxin biosynthesis. Furthermore, MpkA effects ornithine and polyamines biosynthesis depleting the main substrate for siderophore production during iron starvation. In conclusion, MpkA can perform all those functions by fine tuning the balance between the energy invested in various cellular processes required for growth, development and natural product synthesis. It thereby, acts as a regulator to ensure better survival of the fungal cell under a wide variety of conditions.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

176. Regulation of secondary metabolite production in *Fusarium* species by the global regulator *LAE1*. Robert A.E. Butchko[1], Susan P. McCormick[1], Mark Busman[1], Bettina Tudyznski[2] and Philipp Wiemann[2]. [1]National Center for Agricultural Utilization Research, Peoria, IL and [2]Institut für Botanik der Westfälischen Wilhelms-Universität Münster, Schlossgarten 3, 48149 Münster, Germany.

Fusarium species are pathogens of corn and wheat and are capable of producing secondary metabolites that are a food safety concern. These mycotoxins include fumonisins which have known carcinogenic potential and trichothecenes which can inhibit protein synthesis. In addition to these mycotoxins, the potential to produce other secondary metabolites is evidenced by the presence of additional biosynthetic gene clusters for fusarin C, bikaverin and other pigments. Whole genome sequence of *F. verticillioides* revealed the presence of multiple putative secondary metabolite gene clusters, including those that contain polyketide synthase genes as well as nonribosomal peptide synthase genes. An understanding of the transcriptional regulation of these secondary metabolite gene clusters could aid in developing methods to control mycotoxin contamination of food. A global regulator of secondary metabolite gene clusters has been characterized in *Aspergillus* species (*LaeA*) and is conserved in *Penicillium* as well as *Fusarium* species. In *F. fujikuroi*, *LAE1* has been shown to regulate secondary metabolite gene expression. Here we characterize the conservation of this global regulation mechanism in other *Fusarium* species. Homologs of *LAE1* are present in *F. verticillioides*, *F. oxysporum* and *F. graminearum*. Deletion of *LAE1* in *F. verticillioides* results in repression of multiple secondary metabolite gene clusters. Characterization of *LAE1* deletion mutants in *F. graminearum* and *F. oxysporum* is currently being investigated.

177. Characterization of C_2H_2 Zinc Finger Transcription Factors in *Fusarium verticillioides*. Martha Malapi-Wight and Won-Bo Shim. Department of Plant Pathology & Microbiology, Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX 77843-2132

A group of transcription factors (TFs) harboring C_2H_2 zinc finger motif is reported in a variety of organisms, from bacteria to humans. Significantly, 0.7% of the human proteome contains this motif. In fungi, it has been shown that C_2H_2 TFs are involved in a diverse array of biological processes. In *Fusarium verticillioides*, an important maize pathogen, we know that certain TFs play a key role in secondary metabolism regulation, notably transcriptional activation of FUM genes. However, the role of C_2H_2 TFs in *F. verticillioides* secondary metabolism and development remains largely unclear. There are 104 predicted C_2H_2 TFs identified in *F. verticillioides*, and we have been investigating the roles of these TFs to identify pathway-specific regulators of FUM genes. Thus far, we have generated 9 gene-deletion mutants and 1 constitutively expressed mutant, including TF5 and TF9 that encode proteins similar to *Aspergillus nidulans* flbC and *Trichoderma reesei* Ace1, respectively. We were not successful to obtain mutants in 3 TF genes, and it is conceivable that these are essential genes. Mutants showed a variety of different phenotypes in fumonisin B_1 biosynthesis, colony morphology, conidiation, response to oxidative stress, and growth on corn kernels when compared to those of the wild-type progenitor. We are currently in the process of transcriptome analyses and pathogenicity assays to further elucidate the role of C_2H_2 TFs in *F. verticillioides*.

178. Regulation of autolytic hydrolase production in *Aspergillus nidulans*. István Pócsi[1], Tamás Emri[1], Nak-Jung Kwon[2], Melinda Szilágyi[1], Fruzsina Bakti[1], Tünde Pusztahelyi[1], HeeSoo Park[2], Éva Leiter[1] and Jae-Hyuk Yu[2 1] - Department of Microbial Biotechnology and Cell Biology, University of Debrecen, Debrecen, Hungary, e- mail: ipocsi@gmail.com [2] – Departments of Bacteriology and Genetics, University of Wisconsin, Madison, WI, USA, e-mail: jyu1@wisc.edu

In spite of its exceptional biotechnological importance, the autolytic phase of growth is a poorly understood and underexploited area in fungal biology. Nutrient limitation initiates the release of a wide array of cell wall degrading hydrolases (chitinases, glucanases and proteinases) to feed surviving hyphal cells in submerged cultures. Among the autolytic hydrolases produced by the model filamentous fungus *Aspergillus nidulans*, ChiB endochitinase, EngA beta-1,3-endoglucanase, PrtA serine proteinase and PepJ metalloproteinase play a major role in the decomposition of cell wall biopolymers. Importantly, all these autolytic enzymes are regulated by the FluG-BrlA developmental signaling pathway, suggesting that conidiogenesis and autolysis are inherently coupled physiological processes and, hence, share common upstream regulatory elements. The biosyntheses of autolytic hydrolases are sophistically and inter-dependently coordinated to avoid either the early and uncontrolled decomposition of intact hyphae or the inefficient degradation of cell wall biopolymers. Considering cell wall constituents, the enzymatic disruption of the polysaccharide (chitin, glucan) layers seems to be a decisive step in fungal autolysis because the *chiB* and *engA* gene deletion mutants possessed non-autolytic phenotypes.

179. Selection of reference genes for qPCR analysis of the pathogenic fungus *Trichophyton rubrum*. Tiago Jacob; Nalu Peres; Gabriela Persinoti ; Larissa Silva; Mendelson Mazucatu, Antonio Rossi; Nilce Martinez-Rossi. University of São Paulo e-mail: tiagorjacob@usp.br

Quantitative PCR (qPCR) or real-time PCR is an important technique for the quantitative analysis of gene expression, which accuracy is strongly affected by the stability of reference genes used for data normalization. Recent studies demonstrate the need for previous evaluation of reference genes to be used as normalization in gene expression studies using qPCR as a molecular tool. The objective of this study was to investigate the suitability of eight candidate genes for qPCR data normalization in the pathogenic fungus *Trichophyton rubrum*, submitted to different environmental challenges, such as drug exposure, interaction with human nail or skin, heat shock, and growth in different culture medium (malt extract, keratin, and minimal media). The expression stability of the genes *18S rRNA* (18S ribossomal RNA), *gapdh* (glyceraldehyde 3-phosphate dehydrogenase), *act* (actin), *chs1* (chitin synthase), β-*tub* (tubulin beta chain), *top2* (DNA topoisomerase II), *rpb2* (DNA-dependent RNA polymerase II), and *atpD* (ATP synthase subunit beta) was analyzed with the BestKeeper, geNorm and NormFinder programs, revealing that *rpb2* and *chs1* genes are the most appropriate reference genes. These findings will allow further analysis of *T. rubrum*, as well as other dermatophytes, gene expression under a wide range of conditions with improved accuracy and reliability.

180. Azole drug species-dependent responses of the transcription factor AtrR in *Aspergilli*. Ayumi Ohba[1], Kiminori Shimizu[2], Takahiro Shintani[1], Susumu Kawamoto[2], Katsuya Gomi[1]. [1]Graduate School of Agricultural Science, Tohoku university, Japan [2]Medical Mycology Research Center, Chiba University, Japan

It has hitherto been shown that overexpression of a transcription factor gene (*atrR*) resulted in increased azole drug resistance and also in upregulation of gene expression of PDR-type ABC transporters in *Aspergillus oryzae*. In contrast, deletion of *atrR* led to downregulation of ABC transporter gene expression and consequently resulted in significant increase in azole drug susceptibility. Previously, we revealed that expression of *atrR* and three PDR-type ABC transporter genes (*atrA*, *atrF*, and *atrG*) are upregulated by miconazole in *A. oryzae* wild-type strain. In this study, we investigated the expression profiles of *atrR* and the PDR-type ABC transporter genes in the presence of other azole drugs. The *atrR* and PDR-type ABC transporter genes (at least *atrA*, *atrF*, and *atrG*) were upregulated by clotrimazole, but not by itraconazole, ketoconazole, or fluconazole, although deletion of *atrR* resulted in significant increase in itraconazole and fluconazole susceptibility. This suggested that the transcription factor AtrR shows different responses dependent on azole drug species. To identify the target genes regulated by AtrR in the presence of different azole drug, we performed DNA microarray analyses of wild type and *atrR* disruptant, which will be presented. In addition, expression profiles of the *atrR* ortholog and ABC transporter genes in several azole drugs were also examined in human pathogenic fungus, *A. fumigatus*.

181. Effect of primary metabolism on secondary metabolite production in *Aspergillus terreus.* Markus Gressler[1], Christoph Zaehle[2], Kirstin Scherlach[2], Christian Hertweck[2], and Matthias Brock[1] [1]Junior Research Group Microbial Biochemistry and Physiology; [2]Department Biomolecular Chemistry Leibniz Institute for Natural Product Research and Infection Biology (Hans Knoell Institute); D-07745 Jena; Germany; markus.gressler@hki-jena.de

Genome sequencing has shown that *Aspergillus terreus* has the potential to produce a great variety of different natural products. Although several metabolites have been identified, it can be assumed that the potential to produce secondary metabolites is much larger than currently known. Several strategies have been developed to discover new metabolites produced by filamentous fungi. Besides the use of epigenetic modifiers or co-cultivation experiments, targeted overexpression of putative transcription factors provides a promising tool to activate silent gene clusters. Here, we investigated the expression of the only complete PKS-NRPS hybrid gene present in the genome of *A. terreus*. Since overexpression of a putative transcriptional activator adjacent to the PKS-NRPS gene did not activate gene transcription, we constructed a *lacZ* reporter to screen for naturally inducing conditions. Results revealed that expression was activated in the presence of several amino acids at alkaline pH. However, glucose mediated carbon catabolite repression remained as the dominating inhibiting factor. When the adjacent transcription factor, which failed to induce PKS-NRPS expression in initial experiments, was expressed under naturally non-inducing, but also non-repressing conditions, activation of the PKS-NRPS gene was observed. Thus, factors involved in regulation of primary metabolism can override activating effects from cluster specific transcription factors. Finally, product identification revealed that the gene cluster is responsible for producing metabolites of the fruit rot toxin family.

182. Regulatory networks that control morphology and virulence in *Histoplasma capsulatum*. Sinem Beyhan, Matias Gutierrez, Mark Voorhies and Anita Sil, University of California, San Francisco, San Francisco, CA 94143

Histoplasma capsulatum is a dimorphic fungal pathogen that causes respiratory and systemic infections in humans. *H. capsulatum* switches its growth program from an infectious mold (hyphal) form in the soil to a pathogenic yeast form in mammalian hosts. Infection occurs when hyphal fragments are inhaled by the human host. Once inside the host, the pathogen converts to a budding-yeast form, which survives and replicates within host macrophages. Under laboratory conditions, this morphological switch is recapitulated by changing the temperature of the growth environment from room temperature (25˚C) to stimulate hyphal growth to human body temperature (37ºC) to stimulate yeast-form growth. This observation was utilized by our laboratory to identify three genes (*RYP1,2,3*) that are required for yeast-phase growth in response to temperature. *ryp* mutants grow constitutively in the filamentous mold form even at 37ºC. In wild-type cells, *RYP1,2,3* transcripts and proteins accumulate preferentially at 37ºC. In this study, we utilized whole- genome transcriptional profiling and ChIP-chip (chromatin immunoprecipitation-microarray) analysis to identify targets of Ryp1,2,3. Additionally, we performed coimmuniprecipitation to test whether Ryp1,2,3 form a complex. Our findings suggest that (1) Ryp1,2,3 regulates similar and distinct sets of genes; (2) Ryp2 and Ryp3 physically interact; and (3) a transcription factor FacB, which is a target of Ryp1,2,3, regulates the hyphal-to-yeast transition.

183. Phenotypical and Transcriptional Analysis of Photoconidiation in mutants of the RNAi Machinery of *Trichoderma atroviride.* Nohemi Carreras-Villaseñor, Ulises Esquivel-Naranjo & Alfredo Herrera-Estrella Langebio, Cinvestav Campus Guanajuato. México. ncarreras@ira.cinvestav.mx

Trichoderma atroviride is one of the most used biocontrol agent due to its mycoparasitic activity. Conidia are useful as inocula in the field and greenhouse, therefore the understanding of the switch that determines the entry into conidiation is of great interest. In *Trichoderma atroviride*, conidiation is induced by light and the possible participation of small RNAs in this process has not been explored, as well as the role of the proteins involved in their biogenesis and function, such as Dicer (Dcr), RNA dependent RNA polymerase (RdRP) and Argonaute (Ago). The *T. atroviride* genome encodes two *dicer* homologues. We have obtained single and double mutants of them. Photoconidiation is altered in *dcr2* and double mutants. In contrast with the wild type they do not respond to constant exposure to white light. In addition, we carried out high-throughput mRNA sequencing by SOLiD of samples obtained from wild type, *dcr1*, *dcr2* and *dcr1 dcr2* strains after 60 h of exposure to white light. 1655 genes are differentially expressed in the mutant strains, as compared to the wild type. Two genes that are up- regulated in *dcr2* and *dcr1 dcr2* are *ago1* and *rdr3*. When exposed to constant white light, *rdr3* is altered in photoconidiation, but *ago1* is not. These data suggest that the RNAi machinery, hence sRNAs, is involved in the regulation of development in this *Trichoderma*

http://newsearch.cgm.ftpt.br/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

184. Effects of temperature and water activity on *Fusarium verticillioides FUM* genes expression and fumonisins B production. [1]Irene Lazzaro, [2]Antonia Susca, [2]Giuseppina Mulè, [3]Alberto Ritieni, [4]Adriano Marocco, [1]Paola Battilani [1]Institute of Entomology and Plant Pathology, Università Cattolica del Sacro Cuore, via Emilia Parmense, 84, 29100 Piacenza, Italy. [2]Institute of Sciences of Food Production, CNR, Via Amendola 122/O, 70126 Bari, Italy. [3]Department of Food Science, Università di Napoli Federico 2, 80055 Naples, Italy. [4]Institute of Agronomy and Plant Breeding, Università Cattolica del Sacro Cuore, via Emilia Parmense, 84, 29100 Piacenza, Italy. E-mail: paola.battilani@unicatt.it

Fusarium verticillioides is recognized as the main maize pathogen in the world, causing ear rot. Colonized kernels are commonly contaminated by fumonisins B (FBs), secondary metabolites toxic and carcinogenic to animals and humans. In this work we studied how changing temperature (T=20,25,30°C) and water activity (a_w=0.90, 0.95, 0.99) affects both FBs production and the expression of *FUM2* and *FUM21* genes in two *F. verticillioides* strains (ITEM10027 and ITEM1744) grown 21 d in FB-inducing (Malt Extract Agar) and FB-inhibiting (Czapeck Yeast Agar) liquid media. Gene expression analysis, carried through RealTime (RT)-PCR, and FBs quantification with HPLC, were conducted. Fumonisins were produced by both strains, and ITEM10027 was proved the highest producer (10450 vs 2438 ppb). *FUM2* and *FUM21* were expressed in all studied conditions, with the former around 10 times more expressed than the latter. The peak of expression was observed after 14 and 21 days of incubation respectively for *FUM2* and *FUM 21*. The expression of both *FUM* genes analyzed was more influenced by temperature than by a_w in the studied range. Changes in temperature produced limited variation for *FUM2* expression, while the highest expression for *FUM21* was observed at 25°C. Moreover it was proven that incubation time of fungal pure cultures significantly influenced the two *FUM* genes expression and FBs production level, which resulted straight correlated. Work supported by the Italian Ministry of University and Research, PRIN 2007.

185. The role of mRNA 3' tagging in RNA degradation and the cesation of translation. Igor Morozov, Daniel Rigden, Meriel Jones and Mark Caddick. The University of Liverpool, Institute of Integrative Biology, Biosciences Building, Crown Street, Liverpool, L69 7ZB, UK

Two co-transcriptional modifications, the 5'-cap and the 3'-poly(A) tail are essential to both transcript stability and translation. The poly(A) tail serves as a platform for multifunctional protein complexes which coordinate the interplay between translation and degradation and in many cases translation and the mRNA decay pathways share the same proteins, confirming both physical and functional links. Controlled deadenylation of a transcript's poly(A) tail to ~A15 generally precedes decapping and subsequent 5'-3'degradation and/or exosome-dependent 3'-5' decay. Consequently deadenylation represents a critical control point for both mRNA turnover and translation. However, the mechanisms that underlie the switch between mRNA translation and degradation are far from understood. These processes are fundamental to gene regulation, providing a critical point through which rapid physiological change can be instigated in response to environmental shifts and stress. Recently we identified that the initiation of a transcript's degradation is coincident with its modification by the addition of a CU rich sequence element at the 3' end. Here we describe our recent work in Aspergillus nidulans, which is focused on understanding the key molecular events that both trigger and coordinate the rapid degradation of transcripts, including decapping, sub-cellular compartmentalisation of the RNA and cessation of translation.

186. Identification and characterization of genetic regulatory elements downstream of *veA* controlling mycotoxin synthesis in *Aspergillus nidulans.* Vellaisamy Ramamoorthy and Ana M. Calvo Dept. of Biological Sciences, Northern Illinois University, DeKalb, Illinois, USA

The filamentous fungus *Aspergillus nidulans* produces the mycotoxin sterigmatocystin (ST). This polyketide compound is the penultimate precursor in the aflatoxin biosynthetic pathway found in related species such as *A. parasiticus, A. flavus*, and *A. nomius*. In *A. nidulans* the genes involved in ST biosynthesis are clustered in chromosome IV. This gene cluster is regulated by the *aflR*, encoding a specific transcription factor that binds to the promoters of ST genes activating their transcription. Expression of *aflR* is positively regulated by the *veA* gene, encoding a global regulatory protein that controls secondary metabolism and morphogenesis. In order to find additional regulatory genetic elements controlling ST production downstream *veA*, we carried out a chemical mutagenesis in a *veA* deletion strain, unable to express ST genes or produce toxin, and search for revertant mutants that regained the capacity to produce it. The strain used in this mutagenesis also has a mutation in the *stcE* gene, which in the wild type leads to the accumulation of the orange norsonolinic acid (NOR) ST intermediate, facilitating a visual screen. From this mutagenesis we isolated several revertant mutants able to produce NOR, indicating restoration of the ST biosynthetic pathway. Some of these mutations also affected fungal morphogenesis. Several of these revertants, corresponding to different linkage groups, are currently being characterized. Complementation with the *A. nidulans* genomic library followed by sequencing will reveal the identity of these genetic regulatory elements downstream *veA* that are involved in the control of ST biosynthesis.

187. The $Zn(II)_2Cys_6$ binuclear cluster of TamA is required for activation of *gdhA* expression in *Aspergillus nidulans*. Downes, Damien J.[1,2], Brendan L. Taig[1], Sara Lewis[1], Richard B. Todd[2], Meryl A. Davis[1] [1]Department of Genetics, University of Melbourne, Vic, Australia. [2]Department of Plant Pathology, Kansas State University, KS, USA

NADP-dependent glutamate dehydrogenase (NADP-GDH) encoded by *gdhA* is required for the assimilation of alternate nitrogen sources through ammonium in *Aspergillus nidulans*. Previous studies have shown that *gdhA* expression is regulated by three transcription factors: the major transcription activator of nitrogen metabolic genes, AreA, the regulator of leucine biosynthesis, LeuB, and TamA a co-activator of AreA that also interacts with LeuB. At the *gdhA* promoter TamA is the major contributor to gene expression, unlike at most nitrogen assimilation promoters, where TamA plays a minor role in gene activation. We show that mutation of the DNA binding domain in any of AreA, LeuB or TamA reduces activity at the *gdhA* promoter. Significantly this suggests a role for the $Zn(II)_2Cys_6$ binuclear cluster of TamA, which is dispensable for function at other promoters. Using fragments of the *gdhA* promoter fused to the *lacZ* reporter gene we identified two core regulatory regions in the promoter. One regulatory region contains a potential site of action for TamA close to putative binding sites for both AreA and LeuB. At the second regulatory site LeuB acts independently as a modulated activator/repressor to mediate glutamate feedback regulation.

188. The velvet gene in *Mycosphaerella graminicola* is associated with aerial mycelium formation, melanin biosynthesis, hyphal swelling and light signaling. Yoon-E Choi and Stephen B. Goodwin. USDA-ARS/Purdue University, West Lafayette, IN, USA.

The Dothideomycete *Mycosphaerella graminicola* causes septoria tritici blotch (STB), one of the most important diseases of wheat worldwide. Despite the negative impact of *M. graminicola* on wheat production, knowledge about its molecular biology is limited. The velvet gene, *veA*, is a key regulator of diverse cellular processes in many fungi. However, its function in the Dothideomycetes, the largest class of plant-pathogenic fungi, is not known. To test the hypothesis that conserved functions extend to the Dothideomycetes, a *veA*-homologous gene, *MVE1*, was identified and gene-deletion mutants were generated in *M. graminicola*. All of the mutants exhibited consistent pleiotrophic phenotypes, indicating the involvement of *MVE1* in multiple signaling pathways. Δ *mve1* strains displayed albino phenotypes with significant reductions in melanin biosynthesis and less production of aerial mycelia on agar plates. In liquid culture, Δ*mve1* strains showed abnormal hyphal swelling, which was suppressed completely by osmotic stress or lower temperature. In addition, *MVE1* gene deletion led to hypersensitivity to shaking, reduced hydrophobicity, and blindness to light-dependent stimulation of aerial mycelium production but there was no effect on pathogenicity. Therefore, the light-signaling pathway associated with *MVE1* does not appear to be important for STB disease. Our data suggest that *MVE1* plays crucial roles in multiple key signaling pathways and is associated with light signaling in *M. graminicola*.

189. Histone H3 de-methylases are involved in regulating primary and secondary metabolism. Agnieszka Gacek[1], Yazmid Reyes-Domínguez[1], Michael Sulyok[2], and Joseph Strauss[1] [1]Fungal Genetics and Genomics Unit, AIT-Austrian Institute of Technology and BOKU University, Vienna, Austria. [2]Christian Doppler Laboratory for Mycotoxin Research, Department IFA-Tulln, BOKU University, Vienna

Opening of chromatin by modification of histone tails is an important process in the synthesis of fungal secondary metabolites (SM). Trimethylation of histone H3 lysine 9 (H3K9me3) and occupancy of heterochromation protein-1(HepA) at this modification site are important marks of transcriptionally silent heterochromatin. In this work we investigate the role de-methylation of H3K9me3 plays in regulating secondary metabolism in *Aspergillus nidulans*. Our Chromatin Immunoprecipitation (ChIP) data provides evidence that both putative Jumonji C-family de-methylases present in the genome are involved in removing the methylation mark from H3K9me3. Deletion of one of the two genes repressed sterigmatocystin (ST) production and the expression of *aflR*, the main regulator of the ST gene cluster. Surprisingly, deletion of both de-methylases restored *aflR* gene expression, but not ST production. Metabolic and transcriptome analysis of the de-methylase mutants suggest that restoration of *aflR* expression is a consequence of de-regulation of primary metabolism, mainly affecting carbon utilization. ST production itself was not restored due to perturbations in primary metabolism presumably affecting precursor provision. Both, de-methylases and LaeA, the conserved global regulator of secondary metabolism, are required to replace the repressing methylation marks on H3 by activating marks.. These results are the first to provide evidence about the role of histone de-methylases in chromatin remodeling, primary metabolism, and secondary metabolism of *A.nidulans.*

190. Withdrawn

191. A conserved role of the putative GEF RicA in *Aspergillus* growth and development. Nak-Jung Kwon and Jae-Hyuk Yu* Departments of Bacteriology and Genetics, University of Wisconsin, Madison WI 53706 USA

The RIC-8 protein is crucial for the GDP/GTP exchange in the absence of GPCRs (G protein coupled receptors) or other GEFs in animal. We isolated the *ricA* genes from *Aspergillus nidulans* and *Aspergillus fumigatus*. The RicA protein is highly conserved in fungi, and the two predicted RicA proteins share about 80% aa identity. The *ricA* mRNA constitutively accumulates throughout the lifecycle of both species. Functional studies suggest that *AniricA* and *AfuricA* play a fundamental role in fungal growth and development. The vegetative growth and asexual development in the D*ricA* mutant is partially restored by D*rgsA*, but not by the absence of *sfgA*, *flbA*, *rgsB* or *rgsC*, suggesting that GanB (Galpha) might be RicA¡¯s primary target. In fact, RicA physically interacts with GanB in Y2H. However, a constitutively active *ganB* (Q208L) mutation could not suppress D*ricA*. Overexpression of *pkaA*, a downstream effector of GanB, partially restores growth and development of the D*ricA* mutant. Importantly, despite the total absence of conidiogenesis, *brlA* and *vosA* mRNAs accumulate at normal levels in the D*ricA* mutant. Furthermore, overexpression of *brlA* or *fluG* failed to restore conidiation in D*ricA*. These indicate that commencement of conidiation requires RicA- mediated signal input, which is independent to the central pathway (BrlA, AbaA and Wet) in *A. nidulans*.

192. The calcineurin pathway governs dimorphic transition in the pathogenic zygomycete *Mucor circinelloides.* Soo Chan Lee, Cecelia Shertz, Robert Bastidas, and Joseph Heitman Department of Molecular Genetics and Microbiology, Duke University, Durham, NC.

The calcineurin pathway is conserved from yeast to humans and controls numerous cellular processes. In pathogenic fungi, calcineurin functions in both growth and pathogenesis, which makes calcineurin inhibitors attractive antifungal drug candidates. Interestingly, FK506 drives *Mucor circinelloides* to grow as yeast, which is also observed in anaerobic and high CO_2 growth conditions. *M. circinelloides* has three calcineurin subunit A's (CnaA, CnaB, and CnaC) and one calcineurin B subunit (CnbA). CnaC is highly expressed during anaerobic or FK506 treatment, in which the fungus grows as a yeast. *M. circinelloides* grows filamentously in the absence of FK506 and in aerobic conditions, where the expression of CnaC is lower. The expression of CnaA and CnaB were not altered during the dimorphic switch. Interestingly, an FK506-resistant mutant expressed higher levels of CnaC in the presence of FK506 and displayed hyphal growth. *Rhizopus oryzae* also has three calcineurin A subunit genes with a single calcineurin B subunit. Further analysis revealed that three copies of *cna* might have involved a whole genome duplication in *R. oryzae* and individual gene duplications in *M. circinelloides. Phycomyces blakesleeanus* has a single copy of gene for each subunit. Our results demonstrate that the calcineurin pathway regulates dimorphic transition in *M. circinelloides* and variation in the evolutionary trajectory of the calcineurin pathway has been adapted in zygomycetes.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

193. Regulation of stomatal tropism and infection by light in *Cercospora zeae-maydis*. Hun Kim[1], John Ridenour[1], Larry Dunkle[2], and Burton Bluhm[1]. [1]Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA. [2]Crop Production & Pest Control Research Unit, USDA-ARS, Purdue University, West Lafayette, IN 47907, USA.

The fungal genus *Cercospora*, comprised of over 3,000 named species, is one of the most ubiquitous and destructive groups of plant pathogenic fungi, and incurs extensive damage on staple food crops throughout the world. In this study, the discovery that light was required for *C. zeae-maydis* to infect leaves led to the identification of the putative blue-light photoreceptor *CRP1*. Disrupting *CRP1* via homologous recombination revealed roles in multiple aspects of pathogenesis, including tropism of germ tubes to stomata, the formation of appressoria, conidiation, and the biosynthesis of phytotoxins. *CRP1* was also required for photoreactivation after lethal doses of UV exposure. Intriguingly, putative orthologs of *CRP1* are central regulators of circadian clocks in other filamentous fungi, raising the distinct possibility that *C. zeae-maydis* uses light as a key environmental input to coordinate pathogenesis with maize photoperiodic responses. This study identified a novel molecular mechanism underlying infection through stomata in a filamentous fungus, underscores the critical role light plays in pathogenesis in *C. zeae- maydis*, and highlights the tractability of the maize/*C. zeae-maydis* pathosystem as a model for examining infection via stomata and the integration of host and pathogen responses to photoperiod.

194. The Role of the MADS-box family of transcription factors in *Fusarium verticillioides.* Carlos Ortiz and Won-Bo Shim, Plant Pathology & Microbiology Dept., Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX

MADS-box, a group of transcription factors (TFs) extensively studied in plants, is a superfamily of gene regulators present in every eukaryote. MADS-box TFs bind to the regulatory motif CArG-box of functionally diverse target genes. Little is known of their role in fungi, but MADS-box TFs have been characterized in select ascomycetes and shown to play a role in pathogenicity, cell and fruiting body development, as well as mating. Our research aim is to elucidate the function of two MADS-box TFs in *Fusarium verticillioides*, a filamentous fungus with direct association with ear and stalk rots of corn. The fungus also produces the mycotoxin fumonisin B1 (FB1) which is linked to human and animal illnesses. We generated *mads1* and *mads2* gene knock-out mutants via homologous recombination. On V8 agar, *mads1* mutant produced a purple pigment while *mads2* did not differ from the wild type. When grown on autoclaved corn, *mads1* and *mads2* mutants produced significantly less (<10%) FB1 toxin compared to the wild type. Ear and stalk rot pathogenicity assays showed no significant difference on the mutants' ability to colonize the host. Northern analyses revealed that *mads1* reaches its expression peak at day 6 whereas *mads2* remains constant when grown in defined media. Mutants in the complementary mating type are currently being generated in order to study the effects of these TFs in sexual development in *F. verticillioides*

195. Characterization of novel conidiation-impaired mutants in *Fusarium graminearum*. Amanda J. Warner[1], Peter Horevaj[2], Burton H. Bluhm[2], and Joseph E. Flaherty[1]. [1]Coker College, 300 E. College Ave., Hartsville, SC [2]University of Arkansas, Dept. of Plant Pathology, Fayetteville, AR

In *Fusarium graminearum*, the causal agent of head blight of wheat and several other economically important diseases, the morphological transition from filamentous growth to asexual sporulation (conidiation) is critical for dissemination and infection. In spite of this, very little is known about the genetic regulation of this important developmental process. From genetic screens designed to identify genes regulating specific aspects of morphogenesis in *F. graminearum*, we identified several insertional mutants impaired in conidiation, which variously display a range of loss- and gain-of-function phenotypes. One mutant that fails to produce conidia, designated 8B5, contains an insertion within a putative bi-directional promoter of genes FG_10779 and FG_10780. To further understand the impact of the insertion on gene regulation, genome-wide analyses of gene expression were performed with microarrays [Fusarias520715 Affymetrix GeneChip] on the wild type and 8B5 mutant strain under conditions favorable for asexual development. Interestingly, the effects of this mutation result in elevated expression of both genes, therefore implicating one or both as a possible repressor of asexual development. Additional information will be presented regarding the molecular characterization of the 8B5 mutant, as well as other mutants impaired in the regulation of conidiation.

196. Characterization of Circadian Clock Output Pathways Regulated by Adv-1 in Neurospora Crassa Using Chip-seq. Rigzin Dekhang1, Kristina M. Smith2, Erin L. Bredeweg2, Jillian M. Emerson3, Matthew S. Sachs1, Jay C. Dunlap3, Michael Freitag2, and Deborah Bell-Pedersen1 1Department of Biology, Texas A&M University, College Station, TX; 2Program for Molecular and Cellular Biology, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331; 3Department of Biology, Dartmouth Medical School, Hanover, NH.

Circadian clocks are biological timekeeping mechanisms used by phylogenetically diverse organisms to control the rhythmic expression of genes involved in physiology, metabolism and behavior. In Neurospora crassa the blue light photoreceptor and PAS-domain GATA transcription factor (TF) WC-1 interacts with another PAS-domain GATA TF WC-2 to form the White Collar Complex (WCC). In the clock, the WCC functions as the positive element in the FRQ/WCC oscillator and it signals time-of-day information through the output pathways to control the expression of a large number of clock-controlled genes (ccgs). ChIP-seq of WC-2 identified hundreds of targets of the WCC, including the promoters of 24 TFs. These TFs are thought to regulate the expression of second tier targets of the clock. One of these TFs, ADV-1, is expressed with a circadian rhythm. Inactivation of the adv-1 gene abolishes the circadian rhythm in conidiation, but does not alter the level or activity of the FRQ/WCC oscillator components. Taken together, these data suggest that the ADV-1 functions within an output pathway from the clock. Results from ChIP-seq and RNA-seq to identify the direct and indirect targets of ADV-1 will be discussed.

197. *Trichophyton rubrum* gene expression regulation during development in keratin. Rodrigo Cazzaniga; Nalu Peres; Adriane Evangelista; Pablo Sanches; Henrique Silveira; Márcia Marques; Geraldo Passos; Antonio Rossi; Nilce Martinez-Rossi. University of Sao Paulo. Brazil

Trichophyton rubrum is the most prevalent dermatophyte worldwide, infecting human skin and nails. During growth on keratin the secretion of keratinolytic enzymes shifts the extracellular pH from acidic to alkaline, being an efficient strategy for its successful infection and maintenance in the host. The inactivation of the transcription factor PacC in *T. rubrum* leads to a decreased keratinolytic activity and deficiency in infecting nails in vitro. We identified genes regulated by PacC during growth on keratin in a time-course gene expression analysis between wild type and pacC-1 mutant strains, by cDNA microarrays. During growth on keratin we observed in both strains that the germination was followed by a gradual increase in the extracellular pH. The comparison between wild type and pacC-1 throughout the growth times showed an outstanding difference in the global gene expression profile of *T. rubrum*. The genes identified are involved in several cellular processes; their regulation during keratin degradation and pH sensing may be an important step in dermatophyte infection. Our results also suggest the participation of PacC in the regulation of genes possibly involved in the metabolism of keratin, playing a role in the pathogenicity of this dermatophyte. Financial support: FAPESP, CNPq.

198. Effect of different ecological conditions on secondary metabolite production and gene expression in two mycotoxigenic plant pathogen *Fusarium* species: *F. verticillioides* and *F. equiseti*. Irene Lazzaro[1,2], Susan P. McCormick[1], Mark Busman[1], Paola Battilani[2], Robert A.E. Butchko[1]. [1]US Department of Agriculture, ARS, NCAUR, Peoria, Illinois, USA; [2]Catholic University of the Sacred Heart, Institute of Plant Pathology, Faculty of Agriculture, Piacenza, Italy

The genus *Fusarium* includes many species that are plant pathogens and many produce harmful secondary metabolites including fumonisins and trichothecenes. These mycotoxins can cause disease in animals and have been associated with cancers and birth defects in humans. Many factors influence the production of secondary metabolites in *Fusarium* species; however regulation of secondary metabolite gene expression is not well understood. We are interested in environmental factors that affect secondary metabolite gene expression and production of mycotoxins. Water activity has been shown to affect both fungal growth and mycotoxin production in a variety of fungi. Here we investigate the effect of water activity on toxin production and the expression of the *FUM* genes in the fumonisin producing maize pathogen *F. verticillioides* and the *TRI* genes in the trichothecene producing soil born pathogen *F. equiseti* using quantitative real-time PCR.

199. Circadian Clock Control of MAPK Pathways.Bell-Pedersen, D. Teresa Lamb, Charles Goldsmith, and Lindsay Bennett Department of Biology, Texas A&M University, College Station, TX 77843-3258 email: dpedersen@mail.bio.tamu.edu

About 20% of Neurospora genes are under control of the circadian clock system at the level of transcript accumulation, and the bulk of the clock-controlled mRNAs have peak accumulation in the late night to early morning. These data suggested the existence of global mechanisms for rhythmic control of gene expression. Consistent with this idea, we found that the Neurospora OS pathway, a phosphorelay signal transduction pathway that responds to changes in osmotic stress, functions as an output pathway from the FRQ/WCC. ChIP-seq with known oscillator proteins revealed that phosophorelay/MAPK pathway components are direct targets of the White Colar Complex (WCC), providing a direct connection between the clock and the output pathway. Activation of the OS pathway by the FRQ/WCC oscillator culminates in rhythmic OS-2 MAPK activity, which through time-of-day-specific activation of downstream effector molecules, controls rhythms in several target clock-controlled genes.

200. Role of zinc transcription factors CrzA and SltA in morphogenesis and sterigmatocystin biosynthesis in the fungus *Aspergillus nidulans.* Sourabha Shantappa, Eduardo A. Espeso and Ana M. Calvo Department of Biological Sciences, Northern Illinois University, DeKalb, Illinois, USA

Calcineurin is a protein phosphatase that plays an important role as signaling component mediating cation homeostasis, morphogenesis, virulence and antifungal drug action in pathogenic fungi. Calcineurin regulates nuclear localization and activation of a transcription factor called Crz1p in *Saccharomyces cerevisiae*. In the model filamentous fungus *Aspergillus nidulans*, the two zinc transcription factors *crzA*, homolog of the *S. cerevisiae CRZ1* gene, and *sltA* which has no identifiable *S. cerevisiae* homolog, are known to be involved in homeostasis and cation adaptation. CrzA is necessary for response to the presence of large concentrations of Ca2+ in the ambient, while SltA prevents toxicity caused by other cations such as Na+, K+, Li+, Cs+ and Mg2+, but not by Ca2+. Previous studies have revealed that the absence of CrzA affect fungal growth and development in *A.nidulans*, however its role as well as the SltA role on the biosynthesis of natural products such as mycotoxins remains unknown. In our research, we found that these two transcription factors, CrzA and SltA, are necessary for normal production of sterigmatocystin toxin at varying levels of cation concentrations. Calcium supplementation resulted in a decrease in sterigmatocystin (ST) toxin production in *crzA* deletion mutants. Supplementation of potassium to *sltA* deletion mutants also resulted in decreased levels of ST production. The same phenotype was observed for both types of mutants in either *veA+* or *veA1* genetic background. Furthermore, this study shows the first characterization of the role of *A.nidulans sltA* fungal homologs in morphogenesis. Increased concentrations of potassium drastically reduced asexual and sexual development, as well as radial growth in *sltA* deletion colonies.

201. Roles for CSP-1 in Light and Circadian Clock-Regulated Gene Expression. Nicole Knabe1, Chandrashekara Mallappa1, Kristina M. Smith2, Jillian M. Emerson1, Erin L. Bredeweg2, Fei Yang3, Deborah Bell-Pedersen3, Matthew S. Sachs3, Michael Freitag2 and, Jay C. Dunlap1 1Department of Genetics, Dartmouth Medical School, Hanover, NH, 2Program for Molecular and Cellular Biology, Department of Biochemistry and Biophysics, and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331; 3Department of Biology and Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX

The csp-1 gene encodes a transcription factor. It is induced by blue light (Chen et al. EMBO J. 2009) and is also regulated by the circadian clock (Lambreghts et al. GENETICS 2007). Both the gene and the CSP-1 are expressed with peaks in morning, and using ChIP-sequencing we find CSP-1 to bind to many regions of the genome and to influence the expression of both light- and clock-controlled genes. In this manner CSP-1 acts as a second order clock regulator, serving to transduce clock regulation of gene expression from the core circadian oscillator to a bank of output clock- controlled genes (ccgs) as verified by ccg-luciferase gene fusions.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

202. Potential roles for nonsense mediated decay regulation in the *Neurospora* circadian system. Anke Grunler, Arun Mehra, Patrick Collopy, Jennifer J. Loros, Jay C. Dunlap Department of Genetics, Dartmouth Medical School, Hanover, NH

Circadian clocks are, for the most part, driven by transcription/translation based feedback oscillators. In general, positively acting transcription factors drive expression of negatively acting proteins which in turn inhibit those activators. One layer of biological control is at the post-transcriptional level and involves regulated destruction of messages bearing premature stop codons. Nonsense mediated decay (NMD) is thought to be a surveillance system for incorrectly spliced transcripts. It can also target transcripts that have regulated non-productive splicing. Using mutants obtained from the *Neurospora* knockout collection and menadione screening, we have extended the findings of Compton (PhD thesis, UCSC, 2003), which reported period effects of mutants in *upf-1* and now report that similar period effects are seen with mutations in other genes encoding components of the nonsense mediated decay pathway.

203. Mapping and characterization of the Neurospora Spore killer elements. Thomas M. Hammond[1], David G. Rehard[1], Hua Xiao[1], Bryant C. Harris[1], Tony D. Perdue[2], Patricia J. Pukkila[2], and Patrick K. T. Shiu[1]. [1]Division of Biological Sciences, University of Missouri, Columbia, MO 65211 [2]Department of Biology, University of North Carolina, Chapel Hill, NC 27599

For over 30 years, the Neurospora Spore killers (*Sk*) have been largely known as mysterious DNA elements that span a non-recombining region of 30 cM on chromosome III. In heterozygous crosses between *Sk* and non- *Sk* strains, few non-*Sk* ascospores survive. However, naturally-occurring resistant strains have been found. To elucidate the molecular components of the Neurospora *Sk* system, we took advantage of the ability to rapidly place hygromycin resistance markers at targeted locations with NHEJ (Non-homologous end joining) mutants, for the purpose of mapping the Spore killer resistance gene, *r(Sk-2)*. We are using the knowledge gained by the mapping and characterization of *r(Sk-2)* to identify other key components of the *Sk* system, such as the gene(s) responsible for the spore killing process.

204. Light regulation of fruiting body development in the Basidiomycete *Coprinopsis cinerea*. Navarro-Gonzalez, Monica and Kües, Ursula. Georg-August-Universität Göttingen, Germany

Fruiting body development in the model fungus *Coprinopsis cinerea* is controlled by light, temperature and nutrients as external cues and, internally, by the two mating type loci encoding homeodomain transcription factors and pheromones and pheromone receptors, respectively. The fruiting process is well adapted to the normal day-night rhythm and 25˚C are absolutely essential for induction and completion of development. Copper in the medium as a novel regulator however allows fruiting at 37˚C but deformed mushrooms (dark stipes) are formed even in light, as described at 25˚C for mutants with defects in light response. Altering the mineral composition in the growth medium can overcome defects in light regulation and mutants give rise to normal fruiting bodies at 25˚C in the normal day-night rhythm. Fruiting body formation correlates with laccase production. *C. cinerea* has 17 different laccase genes, many of which are expressed in either cap or stipe tissues at different stages during the fruiting process. Specific enzymes of this multi-copper-oxidase family are identified.

205. Investigating the Role of *EFG1* in the Regulation of Morphology in *Histoplasma capsulatum*. Anthony Myint, Sinem Beyhan, and Anita Sil, University of California, San Francisco, San Francisco, CA 94143

Histoplasma capsulatum is a dimorphic fungal pathogen with two distinct morphologies: a hyphal form important for initial infection and a budding-yeast form important for pathogenesis. The switch between hyphal and yeast forms can be triggered in culture by changing the growth temperature from 25˚C to 37˚C. Our laboratory identified the transcription factor Ryp1, which is required for yeast-phase growth as well as the majority of the normal transcriptional response to growth at 37˚C (Nguyen and Sil, PNAS, 2008, 105(12):4880-5). Interestingly, in Candida albicans, the Ryp1 ortholog Wor1 is required for the switch from white to opaque cells, while the transcription factor Efg1 is required for the reverse switch. We hypothesized that a conserved regulatory network may regulate the yeast-hyphal switch in H. capsulatum. We proposed that H. capsulatum Efg1 may oppose Ryp1 to inhibit yeast-phase growth and promote hyphal growth. H. capsulatum EFG1 is enriched in hyphal cells, while RYP1 is enriched in yeast cells. In yeast cells, ChIP-chip experiments reveal that Ryp1 associates with the Efg1 promoter, and in the absence of RYP1, EFG1 is inappropriately upregulated at 37˚C. Furthermore, RNAi knockdown of EFG1 results in an altered hyphal morphology at 25˚C, and ectopic expression of Efg1 at 37ºC results in inappropriate filamentation. We are currently assessing the gene expression profile of both knockdown and ectopic expression strains. These data will shed light on regulation of morphology by Efg1 in H. capsulatum.

206. The development of genetics and genomics for analysis of complex traits in the model filamentous fungus, *Neurospora crassa*. Charles Hall[1], Christopher E. Ellison[1], Elizabeth Hutchison[1], David Kowbel[1], Juliet Welch[1], Rachel B. Brem[2], John W. Taylor[1], N. Louise Glass[1]. Departments of [1]Plant & Microbial Biology and [2]Molecular and Cell Biology, University of California, Berkeley, CA 94720-3102, USA.

Our goal is to develop and make available to the community a set of strains and tools that will facilitate the rapid identification of genes contributing to quantifiable traits in the filamentous ascomycete *Neurospora crassa*, identify regulatory networks on a genomic scale, as well as provide a data-set useful for population genomics. We have used Illumina short-read sequencing of mRNA to simultaneously identify Single Nucleotide Polymorphisms (SNPs) and quantify gene expression for 109 isolates of *N. crassa* from Louisiana USA. The resulting dense marker map has facilitated the mapping of QTLs by association in our wild population with high resolution. Moreover, as most sequence variation in a gene will result in an altered expression level for that gene, combining QTL analyses of physiological and gene expression traits, based on co-localization of expression QTLs (eQTLs) and QTLs can directly indicate candidate genes. We have also utilized this data to identify regulators and their regulatory networks. By this method we will be able to utilize the genetic, phenotypic, and expression variation within a population of *N. crassa* to annotate thousands of previously uncharacterized genes.

207. Iron and Gene Expression: characterization of a GATA SRE transcription factor from *Phanerochete chrysosporium* in *Neurospora crassa.* Canessa, P., Muñoz, F., Olivares-Yáñez, C. and Larrondo, L.F., Depto Genética Molecular y Microbiología, Fac Cs Biológicas, Universidad Católica de Chile.

Iron is an essencial biological element, but toxic in excess. Therefore, iron homeostasis is actively controlled and genes involved in its acquisition are under tight regulation. Iron has been shown to play important roles in lignocellulose degradation by both white and brown-rot fungi. In order to understand the extent of iron regulation in *P. chrysosporium* we performed an in silico genome-wide analysis of putative cis iron responsive elements. Among the identified genes several were validated by RT-qPCR and, as predicted, their mRNA levels were lowered in the presence of iron. Most of the identified genes seem to fall into iron unrelated functions. In order to identify an iron transcriptional regulator, we found in the P. chrysosporium genome an homologue of the N. crassa GATA SRE transcription factor (TF), a major regulator of iron homeostasis. Two types of cDNAs differing in the presence/absence of an intron-module were detected. The functionality of the predicted proteins was evaluated in a N. crassa sre-Delta strain. The two splicing variants could be detected by the addition of a V5-tag and the resulting strains were characterized at the phenotypic and molecular levels. Thus, we confirmed the presence of a functional SRE-like TF in P. chrysosporium. Interestingly, the corresponding gene seems to be absent in some closely related basidiomycetes, suggesting alternative mechanisms mediating the responses to environmental stimuli. Fondecyt 1090513.

208. Identification of transcriptional regulators mediating time-of-day-specific gene expression in the *Neurospora* circadian system. A. Montenegro-Montero, A. González-Vogel, A. Goity, L.F. Larrondo. P. Univ. Catolica de Chile.

Circadian clocks are composed of a central oscillator and two signaling pathways: input pathways convey external signals to the oscillator and output pathways allow it to temporally regulate diverse cellular processes. The ascomycete *N. crassa* has one of the best-understood circadian systems, in which a molecular negative feedback loop involving FRQ and WCC lies at its core. Despite the wealth of knowledge on the molecular basis of some eukaryotic oscillators, little is known about how they can temporally control gene expression and the activity of their targets. Our lab is interested in deciphering these circuits in order to understand how the information is passed on from a central oscillator to regulate rhythmic gene expression. In this work, we describe a set of useful and efficient tools aimed at characterizing transcriptional networks involved in this rhythmic information flow. We are using a luciferase-based high-throughput screening system and a proteomics approach to identify regulators involved in the expression of clock-controlled genes. We have confirmed the rhythmic expression of members of the bZIP family and we are characterizing nuclear protein factors that recognize putative clock cis-acting elements. The combination of these approaches with various tools for gene manipulation, allows for a circadian functional genomics strategy. This systems-oriented approach enables us to study the role of various regulators in *Neurospora's* output pathways, providing a deeper understanding of their biology. FONDECYT 1090513.

209. Comparison of *Neurospora crassa* transcription profiles after mycelia contact with fungi of increasing phylogenetic distance. Christopher F. Villalta[1] and John W. Taylor[2] Department of Plant and Microbial Biology, University of California, Berkeley 1. cvillalta@berkeley.edu 2. jtaylor@berkeley.edu

Neurospora in nature encounters different fungi as it grows on heat-killed plants. To assess the response of *Neurospora* when it encounters other fungi, we determined the differences and patterns of the transcriptional profiles of one *Neurospora crassa* FGSC 2489 individual from the Caribbean clade (NcA) as it encounters fungi of decreasing phylogenetic relatedness. We used RNAseq to characterize transcription for *N. crassa* growing alone and when it encounters itself, another *N. crassa* from the same population, an *N. crassa* from the Indian clade (NcC), an *N. discreta*, and young (1day) or older (4 day) colonies of *Penicillium chrysogenum*. Mycelia from *N. crassa* were collected before and after contact with the other fungi to make, with bioreplication, 39 RNAseq libraries. From the RNAseq data we have found significant differences among the transcription profiles between the various interactions. The largest expression differences are seen when *N. crassa* comes into contact with different individuals of its own species or with the older *Penicillium* colony, which we observed to inhibit the growth of *Neurospora*. We are examining the interactions using fluoresecent microscopy to detect reactive oxygen species and determine if the interactions that we observe are related to heterokaryon incompatibility.

210. Genes differentially expressed during conidiation in *Magnaporthe oryzae.* Kyoung Su Kim and Yong-Hwan Lee Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea

The rice blast caused by *Magnaporthe oryzae* is a major limiting factor in global rice cultivation. *M. oryzae* is a polycyclic pathogen capable of many disease cycles in favorable weather within a period of the crop growing season, which may explain why the pathogen is so destructive to rice in certain areas. Like most fungal pathogens, conidia (asexual spores) of *M. oryzae* play a key role in disease cycle. The spatial dissemination and disease severity leading to the epidemic of rice blast rely on the production of conidia by rounds of the asexual reproduction. A new round of conidiation from infected tissues takes only as little as 3 to 5 days depending on the level of compatibility between the pathogen and host, and environmental condition. Therefore, understanding of molecular mechanisms of conidiation in *M. oryzae* should shed light on development of better strategies for the control of fungal crop disease. However, there has been little progress made towards enhancing understanding on molecular events during conidiation on a whole genome-scale. In this study, we employ an Agilent whole-genome oligonucleotide chip to establish the large-scale expression profiling of *M. oryzae* transcriptome during conidiation. This work reveals that aeration has a high effect on global change in gene transcription; several hundred genes were differentially expressed in conidiation in response to the factor. Many were revealed to encode potential regulators such as transcipriton factors, protein kinase, phosphatases, and etc. A subset of genes induced in conidiation is found to be regulated by a homeobox transcriptional regulator, *MoHOX2*. Functional roles of conidiation genes are predicted with gene ontology terms, and directly characterized with several T-DNA insertional mutants obtained after screening a pool of the library. This study provides a foundation for molecular dissection of the role of conidiation genes, and gaining insights into molecular events controlling conidiation.

211. Carbon metabolism of *Fusarium verticillioides* has effects on fungal development, fumonisin B1 production, and pathogenicity. Hun Kim[1,2], Jonathon Smith[2], Charles Woloshuk[1], and Burton Bluhm[2]. [1]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA. [2]Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA.

It has been postulated that *Fusarium verticillioides* perceives and responds to the breakdown products of starch from maize kernels for colonization and fumonisin B1 (FB1) production. Based on this hypothesis, we created a strain disrupted in *hxk1* that encodes a hexokinase for phosphorylation of hexoses. The resulting mutant was not able to grow on a defined agar medium containing fructose as a carbon source, and also lost the ability of fructose transport into fungal cell. Intriguingly, we found swollen structures from the mutant when grown on a yeast-extract peptone medium supplied with fructose. Related with secondary metabolites, as expected, the mutant produced five-fold reduced amount of FB1 on maize kernels, indicating that HXK1 plays an important role in signal transduction that promotes FB1 production. The disruption of *hxk1* had effects on fungal metabolites during colonization of maize kernels; trehalose production of the mutant was reduced by two-fold compared to the level of wild type. This change in trehalose production probably caused salt stress sensitivity and reduced pathogenicity of the mutant. Together, these results indicate that the carbon metabolisms undergone a change have an effect on fungal development, secondary metabolism, and pathogenicity.

212. Withdrawn

213. Functional characterization of nuclear localization signals in the *Aspergillus nidulans* transcription activator of nitrogen metabolic genes AreA. Richard B. Todd[1], Cameron C. Hunter[1], Kendra S. Siebert[1], Koon Ho Wong[2], Sara Lewis[2], Damien J. Downes[1], James A. Fraser[2], Michael J. Hynes[2] and Meryl A. Davis[2]. [1] Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA. [2] Department of Genetics, The University of Melbourne, VIC 3010, AUSTRALIA.

The *Aspergillus nidulans* GATA DNA-binding transcription factor AreA activates transcription of genes for uptake and metabolism of nitrogen nutrients. AreA accumulates in the nucleus during nitrogen starvation but not in the presence of nitrogen sources. The AreA protein contains five putative classical SV40 Large T Antigen-type nuclear localization sequences (NLSs) and one putative non-canonical bipartite NLS conserved with mammalian GATA4. We are using two approaches to determine which of the putative NLSs are functional. First, we constructed epitope-tagged gene replacement *areA* mutants affected in the NLSs to identify sequences required for nuclear localization. Immunofluorescence microscopy experiments show that at least one of the classical NLSs contributes to nuclear import. However, deletion of all five classical NLSs does not affect utilization of nitrogen sources and does not prevent AreA nuclear localization. Mutation of the bipartite NLS confers inability to utilize alternative nitrogen sources. We are determining the effect of this bipartite NLS mutation individually and in combination with deletion of the five classical NLSs on nuclear localization. Second, we fused DNA sequences encoding the putative AreA NLSs to the Green Fluorescent Protein (GFP) gene and introduced these constructs into *A. nidulans* to determine which of the predicted NLSs are sufficient to direct GFP to the nucleus. We will use UV-fluorescence microscopy to determine the subcellular location of the GFP-NLS fusion protein in the transformants.

214. A reverse and forward genetic clock-screening strategy to identify new circadian regulators in *Neurospora crassa.* Luis F. Larrondo^ Jennifer J. Loros*, Jay C. Dunlap* and Alejandro Montenegro-Montero^ ^- DGMM. P. Universidad Católica de Chile. *- Dept. Genetics, Dartmouth Medical School, USA.

Neurospora circadian rhythms can be indirectly followed by the overt rhythmic appearance of spores (conidial banding). Mutations that affect circadian-gene expression, but not overt rhythmic conidiation, are normally overlooked. To overcome this and other limitations a fully-codon optimized luciferase reporter system for *N. crassa* was developed. By putting this real-time reporter under the control of promoter regions containing circadian elements, rhythms in transcription of *frq* (oscillator component) or clock-controlled genes (*ccgs*) can be easily tracked for over a week. Moreover, by generating FRQ-LUC translational fusion strains, rhythms in FRQ protein can be followed in a semi- quantitative manner. By using a bioluminescence high-throughput screening platform and following a reverse and forward genetic screening strategy and functional genomic tools, we have started to identify interesting candidates affecting either the core oscillator or the output pathways. Thus, we have identified at least one transcription factor that regulates the expression of some *ccgs*, potentially representing a direct link between the WCC (core oscillator) and the downstream output machinery. In addition, we have started to map a new mutant displaying both a period defect and female sterility. As a result, this new experimental setup has started to reveal novel molecular details of the Neurospora clock. Funding: FONDECYT 1090513

215. Expressions of genes for fatty acid metabolism and peroxisomal biogenesis and the hydrophobin production in the farA disruptants of *A.oryzae*. Sharon Marie Garrido[1], Noriyuki Kitamoto[2], Akira Watanabe[1], Takahiro Shintani[1], and Katsuya Gomi[1] [1]Graduate School of Agricultural Science, Tohoku University, Japan; [2]Food Research Center, Aichi Industrial Technology Institute, Japan.

FarA is a Zn_2Cys_6 transcription factor which upregulates genes required for growth on fatty acids in *Aspergillus nidulans.* FarA is also highly similar to the cutinase transcription factor CTF1alpha of *Fusarium solani* . In this study, we examine the implication of FarA in the regulation of genes responsible for the production of hydrophobin proteins which mediate the activity of CutL1 in the degradation of a biodegradable plastic, poly-(butylene succinate-co-adipate) PBSA in *A. oryzae* . Wild-type (WT) and farA disruptants were grown in minimal agar medium with PBSA, and WT showed clear zone around the colony and the presence of HsbA protein while the disruptants did not. However, RolA was both detected in the WT (at higher level) than the disruptant. Furthermore, qRT-PCR and RT-PCR revealed that the expression of *hsbA* and *rolA* genes were significantly reduced in the disruptants compared to WT. In addition to this, expressions of genes such as acyl-CoA dehydrogenase, isocitrate lyase, enoyl-CoA isomerase, carnitine O-acyltransferase and fatty-acyl-coA oxidase for fatty acid metabolism and genes for peroxisomal biogenesis (*pex1, pex11, pex16*) were reduced in the disruptant compared to the WT when grown in minimal medium with oleic acid as a sole C source. These results indicated that FarA may be implicated in the expression of hydrophobin genes and genes for fatty acid metabolism and peroxisomal biogenesis in *A.oryzae* . The data will be relevant for the industrial use of the *A.oryzae* especially in the production of enzymes such as the cutinases/lipases.

216. Regulation of the Meiotic Program in *Candida lusitaniae*. R.K. Sherwood,[1,2] S. Torres[1] and R.J. Bennett. [1,2] [1]Molec. Microbiology and Immunology. [2]Molec. Bio., Cell. Bio., and Biochemistry, Brown University, Providence, RI. Racquel_Sherwood@Brown.edu

Many genes involved in mating and meiosis are conserved across hemiascomycete yeast, including model species *S.cerevisiae*, as well as members of the *Candida* yeast clade. Completion of the mating cycle in sexual species is mediated by meiosis, in which reductive DNA division gives rise to recombinant progeny cells. Recent studies show that *C.lusitaniae* is unusual among *Candida* species in that it undergoes a complete sexual cycle, despite lacking homologs of several genes essential for meiosis in *S.cerevisiae*. In particular, *IME1*, encoding the master meiotic regulator in *S. cerevisiae* is absent from the *C.lusitaniae* genome. In this study, we use genetic and genomic approaches to identify regulators of meiosis in *C.lusitaniae*. We show that homologs of *S. cerevisiae* meiotic genes are induced during *C.lusitaniae* meiosis, suggesting these genes have retained a conserved function. We also constructed mutant strains lacking the serine threonine kinase, *IME2*, in *C.lusitaniae*. Preliminary experiments indicate that *IME2* plays an important role in multiple aspects of sexual reproduction in this species. Overall, we propose that elucidation of conserved and novel meiotic regulators in *C.lusitaniae* will provide further clues as to how different *Candida* species undergo meiosis, despite lacking factors essential to *S.cerevisiae* meiosis.

217. Two classes of new peptaibols are synthesized by a single non-ribosomal peptide synthetase of *Trichoderma virens*. Prasun K. Mukherjee[1,2,] Aric Wiest [3], Nicolas Ruiz [4], Andrew Keightley [5], Maria E. Moran-Diez [2], Kevin McCluskey [3], Yves François Pouchus [4] and Charles M. Kenerley [2]. [1] Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400085, India; [2] Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843; [3] Fungal Genetics Stock Center, University of Missouri-Kansas City, Kansas City, MO 64110.; [4] University of Nantes, Faculty of Pharmacy, MMS – EA 2160, F-44000 Nantes, France; [5] University of Missouri-Kansas City, School of Biological Sciences, Kansas City, MO 64110.

Peptaibols or peptaibiotics are a group of small peptides having a high alpha-aminoisobutyric acid (Aib) content and produced by filamentous fungi, especially by the members of the genus Trichoderma/ Hypocrea. These antibiotics are economically important for their anti-microbial and anti-cancer properties including the ability to induce systemic resistance in plants against microbial invasion. In this study we will present sequences of two classes (11-residue and 14-residue) of peptaibols produced by a biocontrol fungus, *T. virens.* Of the 35 11-residue peptaibols sequenced, 18 are hitherto un-described and all the 53 14-residue sequences described by us here are new. We have also identified a peptaibol synthetase (non-ribosomal peptide synthetase, NRPS) with 14 complete modules from the genome of this fungus and disruption of one single gene (designated as tex2) resulted in the loss of both the classes of peptaibols. We, thus present here an unprecedented case where a single NRPS encodes for two classes of peptaibols. The new peptaibols presented here could have applications as therapeutic agents for the management of human and plant health.

218. Expression pattern of secondary metabolic genes under various culture conditions. M. Umemura[1*], H. Koike[1], M. Sano[2], N. Yamane[1], T. Toda[1], Y. Terabayashi[1], Y. Satou[1], Y. Oosawa[1], K. Abe[3], S. Ohashi[2], and M. Machida[1] [1]Natl. Inst. Adv. Indust. Sci. Tech., [2]Kanazawa Inst. Tech., [3]Tohoku Univ., Japan. [*]umemura-m@aist.go.jp

Fungi produce secondary metabolites which can be good candidates for bioactive agents. It is difficult, however, to obtain the metabolites from cell culture as fungi produce them only under certain particular conditions. If we could design culture condition under that desirable secondary metabolites are produced, such knowledge should lead to discovery of novel secondary metabolites. Toward this goal, we analyzed gene expression distribution of *Aspergillus oryzae* under more than 200 culture conditions using DNA microarray. We used *A. oryzae* as a model although it produces bare secondary metabolites. The culture conditions are mainly classified into three categories: 1. nutrition, 2. time, and 3. chemical addition such as antifungal agents. Each category has sub-classes. Combined with another technology of ours to predict fungal secondary metabolic genes, expression distribution of secondary metabolic genes under each condition was hierarchically clustered. Interestingly, the grouped cluster of gene expression distribution overlaps with the class of culture condition to some extent. The same tendency was observed when selecting the genes considered to concern fatty acid synthesis. This result will lead to the clue to the conditions under that fungi tend to produce more secondary metabolites.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

219. Cpvib-1 is required for cell death triggered by vegetative incompatibility in the plant pathogen *Cryphonectria parasitica*. Rong Mu and Angus L. Dawe, Department of Biology, New Mexico State University, Las Cruces, NM 88003. dawe@nmsu.edu

Genetic regulation of non-self recognition is hypothesized to be a means of limiting the spread of cytoplasmically- transmissable agents such as mycoviruses. Compatible strains will form a stable heterokaryon, while incompatible strains will seal fused compartments that subsequently undergo programmed cell death. In *Neurospora crassa* vib-1 has been highlighted as an transcriptional activator that is required for the expression of downstream effectors. We have explored the role of a putative vib-1 homolog from the plant pathogen *Cryphonectria parasitica*, a model system for mycovirus-host interactions and causative agent of chestnut blight. We have found that when Cpvib-1 was deleted from the wild-type strain EP155, the resulting phenotype included enhanced pigmentation and conidiation, similar to that reported for *N. crassa*. Additionally, the delta-Cpvib-1 strain was significantly compromised for pathogenicity when tested on chestnut tissue. We have further identified a role for Cpvib-1 in mediating the incompatibility response and cell death in *C. parasitica* by testing interactions with strains that contain allelic variations at loci that control compatibility. When Cpvib-1 was deleted from incompatible partner strains, formation of a stable heterokaryon was possible. This study provides the foundation for exploring the role of Cpvib-1 in mediating the processes that restrict mycovirus transfer between colonies.

220. Withdrawn

221. Direct determination of mRNA half-life without disrupting transcription in *Neurospora crassa* reveals roles for uORFs and 3'UTRs in nonsense-mediated mRNA decay. Zhang, Ying and Matthew S Sachs Texas A&M University, College Station, TX, 77843

Nonsense-mediated mRNA decay (NMD) rids cells of mRNAs that contain premature termination codons. NMD in higher eukaryotes employs factors not in *Saccharomyces cerevisiae*, such as the exon junction complex (EJC). We used a pulse-chase procedure to measure *N. crassa* mRNA half-life in wild-type and NMD-deficient strains. 4-thiouracil (4TU) is rapidly incorporated into *N. crassa* RNA; incorporation is blocked by addition of 50-fold excess uracil. We pulsed cells with 4TU, chased with uracil, and purified total RNA and 4TU-containing RNA. *N. crassa* strains containing knockouts of NMD factors showed increased levels of *arg-2* mRNA, which contains an upstream open reading frame (uORF), and *eIF4AIII* (an EJC component) mRNA, which contains a long 3'UTR with a spliced intron. Each mRNA had a longer half-life in *upf1* and *upf2* NMD mutants. We reintroduced these genes into the mutant strains and this reduced overall levels and half-lives of these and other NMD-substrate mRNAs. Luciferase reporters containing wild-type or mutated *arg-2* uORFs, or *eIF4AIII* 3'UTR sequences, were introduced into wild-type and *upf1* strains. The wild-type uORF, but not a mutated uORF, conferred NMD to the reporter. The *eIF4AIII* 3'UTR also conferred NMD and the intron appeared important for control. We are now investigating the roles of *N. crassa* Y14 and Magoh (EJC components) on NMD. The coupling of this pulse-chase methodology with the availability of a comprehensive knockout strain collection should provide the basis for understanding the control mechanisms governing RNA stability in this eukaryote.

222. Genome-wide analysis of *Neurospora crassa* transcripts regulated by the nonsense-mediated mRNA decay pathway. Ying Zhang[1], Fei Yang[1], Mohammed Mohiuddin[2], Stephen K Hutchison[2], Lorri A Guccione[2], Chinnappa Kodira[2], Matthew S Sachs[1]. [1]Texas A&M University, College Station, TX, 77843. [2]Roche 454, Branford, CT, 06405

Nonsense-mediated mRNA decay (NMD) is a surveillance pathway that rids cells of mRNAs that contain premature translation termination codons. It is active in all eukaryotes examined and the core factors are highly conserved. NMD pathways in higher eukaryotes can employ factors that are not present in the yeast *Saccharomyces cerevisiae*, such as components of the exon junction complex (EJC), which has a role in mRNA splicing. The genome of the model filamentous fungus *Neurospora crassa* contains core NMD components as well as EJC components, and, unlike *S. cerevisiae* , many of its mRNAs are spliced. We have established that knockouts of *N. crassa* genes for the NMD components UPF1 and UPF2 lead to the increased stability of specific mRNAs that are NMD substrates. We are using 454 whole transcriptome sequencing to perform studies of transcripts in *N. crassa* strains that are wild-type or deficient in NMD to evaluate at the genome-wide level the changes that occur when this surveillance pathway is eliminated. Here we present the results of our comparative analysis of the whole transcriptome data from wild type and knockout *N. crassa* strains and provide further evidence for the extent and complexity of NMD in regulating transcript metabolism. For example, in the mutant strain, approximately 15% of mRNAs for predicted proteins are at least two-fold up-regulated, and there are a large number of novel exons in the transcriptome.

223. Nitric oxide (NO) is a morphogenetic signal in fungi. Ana T. Marcos*, Thorsten Schinko#, Joseph Strauss#, David Cánovas* *Departamento de Genética, Facultad de Biología, Universidad de Sevilla, Spain, davidc@us.es; #Fungal Genetics and Genomics Unit, AIT-Austrian Institute of Technology and BOKU University, Vienna, Austria, joseph.strauss@boku.ac.at

Nitric oxide (NO•) is an important signalling and defence molecule in higher eukaryotes, including plants and mammals. We recently showed that in A. nidulans this short-lived nitrogen oxide radical is generated during the nitrate assimilation process, and that detoxification by flavohemoglobin proteins FhbA and FhbB, which are co-regulated with the nitrate pathway, is required to protect nitrate- and nitrite reductase from nitrosative inactivation under elevated NO• conditions (1). We here report the effect of nitric oxide donors and of mutations in fhbA and fhbB on developmental processes in two fungal genera. In response to external and internal signals, all fungi undergo developmental programs to form specialized structures and in A. nidulans, there is a fine balance between asexual (conidiation) and sexual development. We have found that addition of the NO•-releasing compound DetaNONOate reduced asexual development in A. nidulans. On the other hand, the formation of sexual structures is increased after DetaNONOate supplementation in several fungal species, including species from Aspergillus and Neurospora. (1) Schinko et al. (2010). Transcriptome analysis of nitrate assimilation in Aspergillus nidulans reveals connections to nitric oxide metabolism. Mol. Microbiol. 78: 720-738.

Cell Biology

224. Regulation of Septins assembly by Rts1 during *Candida albicans*. morphogenesis. [1]David Caballero-Lima, [2]Alberto Gonzalez-Novo, [1]Pilar Gutierrez-Escribano, [1]Carmen Morillo-Pantoja, [2]Carlos R. Vazquez de Aldana and [1]Jaime Correa- Bordes. 1 Ciencias Biomedicas. Facultad de Ciencias, Universidad de Extremadura, Avda Elvas sn, Badajoz 06071, Spain. Phone: +34924289300 ext 86874, Fax: +34924289300, e-mail: jcorrea@unex.es 2 Inst. Microbiologia Bioqca. Dpto. Microbiologia y Genetica. CSIC/Universidad de Salamanca.

Inhibition of cells separation is characteristic of hyphal growth in *Candida albicans*. This inhibition is dependent on Sep7 phosphorylation by the hyphal-specific cyclin Hgc1, which regulate dynamic of the septin ring. Here, we show the role of Rts1, a regulatory subunit of PP2A phosphatase, in septin ring regulation in yeast and hyphal growth. In yeast, Rts1-Gfp translocates transiently from the nucleus to the bud neck after actomyosin ring contraction and is mainly located at the daughter side of the septin collar. In accordance with this, yeast cells lacking RTS1 fail to split septin ring properly during citokynesis. Whereas in wild-type cells the septin collar divides in two septin rings of similar diameter, rts1 mutant cells ring at the side of the daughter is significantly wider than the mother. This difference correlates with buds having a bigger size than the mothers. Moreover, disassembly of septins rings is compromised at the end of citokynesis and they persist for several cell cycles. During hyphal induction, rts1 mutant cells show a pseudohyphal-like growth. Interestingly, septin rings were misshapen and some longitudinal septins filaments were observed at the tip of the apical cell. In addition, aberrant septins structures could be seen at the cortex of yeast and hyphae. Furthermore, all septins were analyzed by SDS-PAGE, finding there is an increase in Sep7 phosphorylation levels. These results indicate that Rts1 is necessary for the normal assembly of septins structures in *Candida albicans*.

225. Septin-mediated morphological transitions during plant infection by the rice blast fungus. Yasin F Dagdas and Nicholas J Talbot Biosciences, College of Life and Environmental Sciences, Geoffrey Pope, University of Exeter, Stocker Road, Exeter, EX4 4QD, UK

Magnaporthe oryzae is the causal agent of rice blast disease, which is a serious threat to global food security. Global yield losses caused by the fungus are approximately $6 billion per annum. *M. oryzae* undergoes several morphogenetic transitions during plant infection and tissue colonization and this plasticity is important for pathogenicity. However, it is not known how cell shape is controlled during the infection-associated developmental phases exhibited by the fungus. Septins are small GTPases that are cytoskeletal elements known to control various morphogenetic events in both yeasts and filamentous fungi. We reasoned that septins might be important regulators of infection-related development in Magnaporthe. We generated an isogenic set of five mutants, each differing by a single septin gene. We also constructed strains of *M. oryzae* expressing fluorescently-labelled septins to facilitate live cell imaging of septin hetero- oligomeric complexes during plant infection. We observed that all septin mutants are defective in pathogenesis and exhibit abnormal cell shapes. Septins form a wide variety of structures, including collars, rings, filaments, bars and patches. The *sep3* mutant is completely non-pathogenic and also appears to be defective in cell cycle progression, the cell integrity pathway and actomyosin ring formation. We also speculate that septins may act as diffusion barriers during appressorium development, based on abnormal localisation of appressorium-specific gene products in septin-deficient mutants. An investigation into the role of septins during plant pathogenesis will be presented.

226. Biomolecular fluorescence and transcriptomics reveal physical and transcriptional interactions among ammonium transporters and signaling components of *Ustilago maydis*. Jinny A Paul, Michelle Barati, and Michael H. Perlin. Department of Biology, Program on Disease Evolution, and Kidney Disease Program, University of Louisville, Louisville, Kentucky, USA

Many pathogenic fungi utilize their ability to switch from budding to filamentous growth to cause disease. One of the cues for such dimorphic switch is the availability of nutrients. In the presence of abundant carbon and nitrogen source, fungal cells of *Saccharomyces cerevisiae* and *Ustilago maydis* grow by budding. However, under conditions of nitrogen limitation, the cells undergo filamentous growth. Ammonium transporters genes like MEP1, 2 and 3 in *S. cerevisiae* and Ump1 and Ump2 in *U. maydis* are important for uptake of ammonium as a nitrogen source. Moreover, Mep2 and Ump2 are capable of sensing low ammonium availability and transmitting this signal as a trigger for the dimorphic switch. In *S. cerevisiae*, Mep2, a sensor of ammonium availability, interacts with signal transduction pathways that activate downstream effectors controlling gene expression. Bimolecular fluorescence complementation was used to determine the physically interacting partners of the *U. maydis* Ump2. Using such an assay we were able to determine conclusively that Ump2 interacts with its paralog, Ump1 (a low affinity ammonium transporter), and it also interacts with Rho1, a component of the signaling cascade that regulates polarized growth. Our experiments have also shown that when grown in carbon and nitrogen-replete conditions, *U. maydis* cells over-expressing the *ump2* gene, show filamentous growth; in contrast, wild type cells under these conditions reproduce by budding. Transcriptome analyses comparing haploid cells deleted for the *ump2* gene with cells over-expressing the *ump2* gene, under both nitrogen starved and carbon and nitrogen abundant conditions were conducted. Here the goal was to explore possible roles for Ump2 in regulating transcription levels, as well as the possibility of coordinate regulation of the *ump2* gene along with those for other components of signaling pathways. Preliminary analyses revealed changes in the expression of some genes shown in earlier studies to be involved in pathogenicity to corn plants such as *egl1*, *rep1* and *kpp6*.

227. Dynamics of horizontal chromosome transfer in *Fusarium oxysporum*. Shermineh Shahi[1], Erik Manders[2] and Martijn Rep[1] [1]Plant Pathology and [2]Centre for Advanced Microscopy, Swammerdam Institute for Life Sciences, University of Amsterdam, P.O. Box 94215, 1090 GE Amsterdam, The Netherlands.

Fusarium oxysporum (Fo) is known as a diverse and widely dispersed pathogenic species complex showing a broad host range, reaching from plants to humans and other mammals. Comparative genomics revealed lineage-specific (LS) genomic regions in *Fusarium oxysporum f. sp. lycopersici (Fol)* that include four entire chromosomes and account for more than 25% of the genome. At least two LS chromosomes can be transferred horizontally to non-pathogenic *Fo* strains, resulting in pathogenicity towards tomato in the recipient. To unravel the mechanics of horizontal chromosome transfer we will use the live-cell fluorescence system developed by Ruiz-Roldan *et al*. (2010) to first observe nuclear dynamics during hyphal fusion events between chromosome donor and acceptor strains. Subsequently, the split-GFP technique will be established to (i) examine temporal and spatial distribution of hyphal fusion between different donor and acceptor strains and (ii) determine whether LS chromosomes are transferred *via* nuclear fusion or exit the donor and enter the acceptor nucleus. Reference: Ruiz-Roldan M. C., Köhli M., Roncero M. I. G., Philippsen P., Di Pietro A., Espeso E. A. (2010) Nuclear Dynamics during Germination, Conidiation, and Hyphal Fusion of Fusarium oxysporum; Eukaryotic Cell 9: 1216-1224

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

228. Cryptococcal WASp homolog Wsp1 functions as an effector of Cdc42 and Rac1 to regulate intracellular trafficking and actin cytoskeleton. Gui Shen[1], J. Andrew Alspaugh[2] and Ping Wang[1,3,4],[1]Research Institute for Children, Children's Hospital, New Orleans, Louisiana USA; [2]Departments of Medicine and Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina USA; and Departments of [3]Pediatrics and [4]Microbiology, Immunology and Parasitology, Louisiana State University Health Sciences Center, New Orleans, Louisiana USA,gshen@chnola-research.org

Cryptococcus neoformans is an opportunistic human fungal pathogen that infects mainly immunocompromised patients, causing meningoencephalitis. It is thought to secrete virulence associated factors into its environment, which indicates proteins involved in intracellular transport may be ideal anti-fungal targets. We have recently characterized a novel endocytic protein, Cin1, and found that Cin1 interacts with Wsp1, a WASP homolog, and Cdc42, a Rho family GTPase. We found that Wsp1 also plays an important role in morphogenesis, intracellular transport, and virulence of the fungus. Additionally, we found that Wsp1 tagged with DsRed co-localizes with the GFP-actin and the GFP-Arp2, suggesting Wsp1 has a conserved role in activating the Arp2/3 protein complex. Both the basic and the GTPase binding domain of Wsp1 appear to play an auto-inhibitory role, similar to mammalian WASp proteins. Activation of Wsp1 by Cdc42 resulted in plasma membrane distribution, suggesting a role in exocytosis, and loss of Cdc42 function caused disappearance of actin cables in the wsp1 mutant, indicating that Wsp1 is an effector of Cdc42. We also provided evidence demonstrating that Wsp1 is an effector of another Rho GTPase, Rac1, in the regulation of vacuolar morphology. Our combined data showed that functions of Wsp1 in intracellular trafficking, vacuole morphogenesis, and actin cytoskeleton are mediated through its role as an effector of both Cdc42 and Rac1. The knowledge gained may extend the current understanding of WASp and Rho-family small GTPases in other eukaryotic organisms.

229. Elucidation of functional domains in the Aspergillus nidulans conidiation regulatory factor FlbB. Oier Etxebeste1, Marc S. Cortese1, Erika Herrero-Garcia2, Aitor Garzia1, Eduardo A. Espeso2 and Unai Ugalde1. 1 Dept. of Applied Chemistry, Faculty of Chemistry, University of The Basque Country. Manuel de Lardizabal, 3, 20018, San Sebastian, Spain. 2 Dept. of Cellular and Molecular Medicine, Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu, 9, 28040, Madrid, Spain.

Asexual development in the model fungus Aspergillus nidulans comprises the successive generation of specialized cell types, collectively known as the conidiophore. The foot-cell, stalk, vesicle, metulae and phialides allow the production of dispersive propagules called conidia. These cellular transformations are induced at the molecular level by a group of proteins among which the bZIP type transcription factor FlbB plays stage-specific key roles. Previous studies described an apical and nuclear localization for FlbB during vegetative phase and conidiophore development, identified an apical interaction partner called FlbE and demonstrated that FlbB mediates the activation of the central conidiation pathway regulator brlA, jointly with the transcription factor FlbD. In this study, we have conducted a bioinformatic analysis of FlbB domains and ascribed putative functions based on structure searches and motif conservation analyses. Based on these assumptions, we generated a set of point mutations within FlbB and systematically analyzed their effects through phenotypic and molecular tracking methods. Furthermore, we identified genes regulated by FlbB activity through genomic and proteomic approaches, which indicated that the scope of FlbB regulation spans as far as the expression enzymes in primary metabolism as well as factors involved in cellular integrity. Overall, these results validate bioinformatic approaches for the design of experiments focused on the elucidation of FlbB functional domains and the molecular mechanism it follows for the control of conidiation and cell integrity.

230. Stage-specific functions of transcription factors in Aspergillus nidulans asexual development are defined through programmed protein complex modules. Aitor Garzia1, Julio Rodríguez-Romero2, Marc S. Cortese1, Reinhard Fischer2, Eduardo A. Espeso3 and Unai Ugalde1. 1 Dept. of Applied Chemistry, Faculty of Chemistry, University of The Basque Country. Manuel de Lardizabal, 3, 20018, San Sebastian, Spain. 2 Karlsruhe Institute of Technology, Institute for Applied Biosciences, Department of Microbiology, D-76187 Karlsruhe, Germany 3 Dept. of Cellular and Molecular Medicine, Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu, 9, 28040, Madrid, Spain.

Fungal hyphae are capable of generating diverse cell types, and can thus be considered as pluripotent cells. The formation of mitospores on emergence to the air involves the participation of hyphal factors known as Upstream Developmental Activators (UDAs), which coordinate the genesis of new cell types, and eventually activate a sporulation- specific pathway (Central Developmental Pathway; CDP) ultimately resulting in the formation of spores. Recent studies have shown that UDAs carry out diverse roles at distinct stages of development (Garzia et al., 2010 and references therein). Thus, the bZip-type UDA FlbB is found associated to another UDA FlbE at the Spitzenkörper, forming an apical complex as a signal-processing center. In the nucleus, however, FlbB activates flbD expression and both factors then jointly activate the first member of the CDP brlA transcription. The combined results indicate that programming of multiple tasks by UDA's at different stages of development likely involves the formation of modular protein complexes. Using the high resolution and sensitivity afforded by RNA-Seq, we have identified a substantial number of novel transcripts that are controlled by UDAs at different stages of development.

231. Vacuole homeostasis by a balance of membrane fission and fusion. Lydie Michaillat, Tonie Baars and Andreas Mayer. Department of Biochemistry, University of Lausanne, 1066 Epalinges, Switzerland

Many organelles exist in an equilibrium between fragmentation and of fusion which determines their size, copy number and shape. Yeast vacuoles rapidly (<1 min) fragment into up to 15 smaller vesicles under hypertonic stress and they coalesce into one big organelle upon nutrient limitation or hypotonic stress. Vacuoles also fragment and fuse in response to the cell cycle and nutrient availability. We have screened mutants defective in vacuole fragmentation and we have reconstituted the fragmentation reaction in vitro with isolated organelles. The in vitro reaction faithfully reproduces in vivo vacuole fragmentation. By a combination of in vivo and in vitro approaches we show that surprisingly vacuole fragmentation (membrane fission) depends on several components of the membrane fusion machinery, e.g. specific SNAREs. In addition, we show that the TOR signaling pathway, which is regulated by upon starvation, induces coalescence of the vacuoles into one big organelle under these conditions. We discovered that TOR positively regulates vacuole fragmentation whereas it has no influence on vacuole fusion. The resulting selective downregulation of fragmentation explains the decrease of vacuole number and the increase of their size under starvation conditions. Our combined in vivo and in vitro approaches have the potential to elucidate the regulatory interplay of membrane fusion and membrane fission machinery that determine organelle structure.

Published by New Prairie Press, 2017

232. The *Trichoderma* LaeA orthologue LAE1 identifies new targets of epigenetic regulation in fungi. Razieh Karimi[1], Jin Woo Bok[2], Markus Omann[1], Susanne Zeilinger[1], Rita Linke[1],Bernhard Seiboth[1], Scott Baker[3],Nancy P. Keller[4],Christian P. Kubicek [1]. [1]Institute of Chemical Engineering, University of Technology of Vienna, Vienna, Austria. [2]Department of Medical Microbiology, University of Wisconsin-Madison, USA. [3]Fungal Biotechnology Team, Chemical and Biological Process Development Group, Pacific Northwest National Laboratory, USA. [4]Department of Medical Microbiology, Department of Bacteriology, University of Wisconsin-Madison, USA.

Trichoderma reesei is an industrial producer of enzymes of lignocellulosic polysaccharide degradation to soluble monomers that can be fermented to biofuels. The genes encoding these enzymes in *T. reesei* have been shown to be clustered in the genome together with genes encoding secondary metabolites. The global regulation of these biosynthetic pathways is only poorly understood. Recently, the velvet complex containing VeA and several other regulatory proteins was shown to be involved in global regulation of secondary metabolism and differentiation in *Aspergillus nidulans*. Here, we report on the *T. reesei* LaeA orthologue LAE1, which was identified by a phylogenetic approach, and which was functionally characterized by using a knock-out strain (*lae1*) and several overexpressing strains (OE*lae1*). Using a transcriptomic approach, we identified functional gene categories that respond to modulations in LAE1 activity. Most strikingly, we observed an absolute dependence of expression of all known cellulase genes on *lae1*, and their strong overexpression in OE*lae1* strains. This provides an experiment-based explanation of the advantage for clustering of cellulases in the genome of *T. reesei*. In addition, we detected a regulatory role of *lae1* in expression of the 20-and 14-residue peptaibol synthetases (*tpc1*and *tps1*), phenotypic characteristics such as pigmentation and sporulation, formation of hydrophobins, heteroincompatibility genes and G-protein coupled receptors. Our data with *T. reesei* extend the known roles of epigenetic regulation by LaeA to processes related to interaction of the fungus with its environment.

233. Functional analysis of the AAA ATPase AipA localizing at the endocytic sites in the filamentous fungus *Aspergillus oryzae*. Yujiro Higuchi, Manabu Arioka, Katsuhiko Kitamoto. Department of Biotechnology, The University of Tokyo, Japan.

We explored novel components involved in endocytosis by the yeast two-hybrid (YTH) screening using AoAbp1 (*Aspergillus oryzae* actin binding protein) as bait. A gene named *aipA* (AoAbp1 interacting protein) which encodes a putative AAA (ATPases associated with diverse cellular activities) ATPase was obtained. Further YTH analyses showed that 346-370 aa region of AipA interacted with the two SH3 domains of AoAbp1. AipA interacted with AoAbp1 *in vitro*, and in *A. oryzae* EGFP-AipA co-localized with AoAbp1-mDsRed at the tip region, suggesting that AipA functions in endocytosis. Although *aipA* disruptants did not display any phenotypic alteration in several culture conditions, *aipA*-overexpressing strains showed defective growth and the aberrant hyphal morphology. Moreover, we generated strains which have mutations of either $aipA^{K542A}$ or $aipA^{E596Q}$. These mutations were introduced in the ATPase domain of AipA and would cause defect in the ATPase activity. In contrast to the strain overexpressing WT *aipA*, the growth of mutated strains was normal, suggesting that ATPase activity is important for the function of AipA. Furthermore, the *aipA*-overexpressing strain displayed a delay in FM4- 64 transport to Spitzenkorper, whereas the mutated *aipA*-overexpressing strains did not, suggesting that AipA negatively regulates apical endocytic recycling.

234. Characterization of Fluconazole-related Chromosomal duplication in *Cryptococcus neoformans.* Popchai Ngamskulrungroj, Yun Chang and Kyung J. Kwon- Chung Molecular Microbiology Section, Laboratory of Clinical Infectious Diseases, NIAID, NIH, Bethesda, Maryland, USA Email: ngamskulrungrp@niaid.nih.gov

Cryptococcus neoformans, a basidiomycetous yeast, causes opportunistic infection mainly in HIV patients worldwide. Fluconazole (FLC), an antifungal triazole drug, has been the drug of choice for the treatment of cryptococcosis and fluconazole therapy failure cases have been increasingly reported. Recently, an intrinsic mechanism of adaptive resistance to triazoles termed heteroresistance (HR) was characterized in *C. neoformans*. Heteroresistance was defined as the emergence of a resistant minor subpopulation that could tolerate concentrations of FLC higher than the strain's MIC. The lowest concentration of fluconazole at which such a population emerged was defined as its LHF (level of heteroresistant to fluconazole). These resistant subpopulations were found to contain disomic chromosomes (Chr). Only Chr1 was found to be duplicated in the populations that grew at their LHF. However, additional duplications involving Chr4, Chr10 and Chr14 were observed as the drug concentration was increased. The roles of *ERG11*, the major target of FLC, and *AFR1*, an ABC transporter with FLC specificity, were found to be important for Chr1 duplication. However, the factors affecting duplication of the other chromosomes have not yet been identified. Since FLC is known to cause perturbation of the cell membrane and is effluxed by various ABC transporters, nine genes on Chr4 that are putatively associated with such functions were disrupted by biolistic transformation. Regardless of their impacts on FLC susceptibility, disruptions of the homologs *SEY1*, a GTPase, *GLO3* and *GCS1*, the ADP-ribosylation factor GTPase activating protein, reduced the frequency of Chr4 duplication. In addition, deletion of a *YOP1* homolog, which is known to interact with *SEY1* and located on Chr7, also reduced the Chr4 duplication frequency. This suggests that the function of these genes is important for duplication of Chr4 under FLC stress in *C. neoformans*.

235. Identification of New DNA Damage Response Proteins Using a Genetic Screen. Larson, J. R. and Osmani, S. A. The Ohio State University, Columbus, Ohio. larson.315@osu.edu, osmani.2@osu.edu

The cellular response to DNA damage involves many well characterized proteins. However, there are likely still unidentified proteins that play important roles in these pathways. For example, several nuclear pore complex proteins (Nups) are required for resistance to DNA damage via unknown mechanisms. SonB is an essential Nup in *Aspergillus nidulans* and a mutant allele of this protein that confers temperature-dependent DNA damage sensitivity was previously isolated in a genetic screen for suppressors of the temperature-sensitive *nimA1* mitotic kinase mutation (De Souza et al., 2006 Genetics 174, 1881-93). Importantly, subsequent analyses showed that SonB is involved in a novel DNA damage response pathway. No other proteins have yet been linked to this pathway. To identify other proteins involved with SonB we have undertaken a genetic screen for DNA damage-sensitive *nimA1* suppressors and have isolated two previously uncharacterized proteins, AN1902 and a new Nup, AN11115. AN11115-GFP localizes to the nuclear periphery throughout the cell cycle and at mitosis forms several distinct foci similar to another Nup, Pom152. Deletion of AN11115 causes marked temperature-dependent DNA damage sensitivity, similar to SonB mutants. Affinity purification and mass spec analysis of AN11115 identified AN1902 thus linking together the genetic and biochemical data. Further studies will add to our understanding of how the nuclear pore complex and the NIMA kinase are involved in the DNA damage response and allow us to map out this novel DNA damage response pathway. (Supported by a NIH National Research Service Award (T32CA106196) to JRL)

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

236. *Cryphonectria hypovirus 1* ORF-A: Sub-cellular localization of protein p40 and role of p29 as a determinant of virus-induced vesicle accumulation within host cells. Debora Jacob-Wilk, Jie Hu, Pam Kazmierczak and Neal K. Van Alfen. Department of Plant Pathology, University of California, Davis, CA 95616.

Cryphonectria hypovirus 1 (CHV1) causes vesicle accumulation and reduced virulence on its fungal host *Cryphonectria parasitica*. We have used two viral strains (CHV1 and CHV2) to determine what viral component is responsible for vesicle accumulation in the fungal host cells. Sub-cellular fractionations of wild type and viral containing fungal strains show that CHV1 causes vesicle accumulation in different nuclear backgrounds of *C. parasitica*. Vesicles accumulate on the lower fractions of the gradients and have previously been shown to contain viral elements and fungal trans-Golgi network markers. The same vesicle accumulation pattern was not observed upon CHV2 infection of *C. parasitica*. The main difference in the genome organization of these viral isolates resides in open reading frame A (ORF A). CHV1 encodes a 69 kDa polyprotein that undergoes proteolytic processing into p29 and p40, CHV2 encodes a 50 kDa polyprotein that does not undergo proteolytic processing and does not code for a cysteine protease homologous to p29. Therefore, our data suggest that p29 is the viral determinant responsible for vesicle accumulation. The vesicle fractionation protocol previously used to characterize the sub-cellular localization of viral p29 was used to find the sub-cellular localization of p40, the second proteolytic product of p69. Polyclonal antibodies produced against a p40 specific synthetic peptide, detected a viral specific band that localizes to the soluble fractions of *C. parasitica*. Using p29 antibodies we were able to detect the undigested precursor, p69, in the soluble sub-cellular fractions.

237. Comparative analysis of hyphal Ca^{2+} dynamics in three *Fusarium* species and the role of calcium channel genes in the generation of hyphal tip Ca^{2+} pulses. Hye-Seon Kim[1,2], Kirk Czymmek[2] and Seogchan Kang[1].[1]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802,USA; [2]Department of Biological Sciences, University of Delaware, Newark, DE 19716,USA. E- mail: hxk234@psu.edu

Calcium is a universal messenger that directs an array of diverse cellular and developmental processes in response to external stimuli. Pulsatile cytoplasmic calcium ($[Ca^{2+}]_C$) signatures, generated by combined action of several types of channels in the plasma and organellar membranes, are believed to translate external stimuli to specific cellular responses through the well-conserved calcium signaling pathway. However, visualization of subcellular $[Ca^{2+}]_C$ dynamics in yeasts and filamentous fungi has been difficult due to technical challenges associated with probes used for imaging $[Ca^{2+}]_C$. Previously, we transformed two fungi, *Fusarium oxysporum* and *Magnaporthe oryzae*, with Cameleon (YC3.60) and imaged dynamic $[Ca^{2+}]_C$ in relation to specific stimuli and key growth- or development-related events such as branching, septum formation, and cell-cell contact. We successfully expressed Cameleon in the cytoplasm of three other *Fusarium*species, including *F. graminearum*, *F. verticilliodes*, and *F. solani*. A comparison of temporal and spatial dynamics of $[Ca^{2+}]_C$ among *Fusarium* species revealed that all species showed tip high $[Ca^{2+}]_C$ but time-lapse ratiometric analysis revealed apparent species-specific pulsatile patterns. Furthermore, in order to better understand which calcium channels play a role in generating pulsatile $[Ca^{2+}]_C$ signatures, three channel genes were specifically disrupted in *F. graminearum*. Taken together, this study provided important clues on fundamental aspects of subcellular $[Ca^{2+}]_C$ signaling in filamentous fungi.

238. Analysis of *Aspergillus nidulans* homolog of Mozart1, a newly identified spindle pole protein. Tetsuya Horio[1], Takashi Toda[2] and Berl R. Oakley[1] [1]Department of Molecular Biosciences, University of Kansas, Lawrence, KS. [2]Cancer Research UK, London Research Institute, London, UK.

Mozart1 is a small centrosomal protein (82 aa) which was newly identified by high-throughput genome-wide screening of animal cells. It plays an important role in mitosis (Hutchins et al., Science 2010, 328, 593-). A homolog of Mozart1 (*mzt*A) that exhibits about 50% amino acid identity is encoded by a single gene in *A. nidulans*. Tagging MZTA with fluorescent proteins revealed that MZTA localizes to spindle pole bodies indicating that this polypeptide is likely to be an functional homolog of Mozart1. Strains carrying a deletion of *mzt*A were viable but exhibited a weak temperature sensitivity. In *mzt*A deletants, fewer cytoplasmic microtubules were observed suggesting that nucleation of the microtubules is suppressed in the absence of the MZTA. We investigated the functional relationship between MZTA and the γ-tubulin complex proteins (GCPs). Double deletion of *mzt*A and nonessential GCP genes (*gcp*D-F) exhibited a variety of synthetically sick phenotypes. While the absence of these nonessential GCPs does not affect growth significantly, depleting both MZTA and one of these GCPs at the same time caused a significant growth reduction. Our data indicates that the MZTA plays an important role in organizing the microtubules in *A. nidulans* by interacting with γ-tubulin and/or γ-tubulin complex proteins.

239. Gle1 translocates from the nuclear pore complex to the nuclear membrane during mitosis in *Aspergillus nidulans*. Mahesh Chemudupati, Aysha Osmani and Stephen A. Osmani Department of Molecular Genetics, The Ohio State University, Columbus, OH

During mitosis in *Aspergillus nidulans*, a subset of nuclear pore complex proteins (Nups) disperses from the core pore structure. This subset of Nups includes all budding yeast orthologs classified as peripheral Nups, with one exception. The predicted peripheral Nup An-Gle1 is unique in that it remains at the nuclear envelope (NE) during mitosis, an attribute typical of core Nups (Osmani et al. Mol. Biol. Cell, 17, 4946–4961, 2006). Additionally, GFP labeled An-Gle1 is distinctly located at the nuclear membrane surrounding the nucleolus during mitosis (Ukil et al. Mol. Biol. Cell, 20, 2132–2145, 2009). Affinity purification of all known Nups in *A. nidulans* identified a protein that co-purified with An- Gle1. This protein, AN0162, has a predicted C-terminal transmembrane domain. Endogenously GFP-tagged full-length AN0162 locates to the nuclear membrane throughout mitosis in a manner identical to An-Gle1 as does the GFP-tagged C-terminal half of the protein. Further analysis showed that AN0162 is responsible for tethering An-Gle1 to the NE specifically during mitosis, but not during interphase. This is the first known instance of a protein targeted to the NE by two different mechanisms. An-Gle1 is targeted to NPCs during interphase, but during mitosis An-Gle1 is targeted to the nuclear membrane by associating with AN0162. Our findings thus agree with the categorization of An-Gle1 as a peripheral Nup. This expands our knowledge of how proteins can be targeted to the NE in a regulated cell-cycle dependent manner. Supported by National Institutes of Health grant GM042564

240. Identification of a new transmembrane nuclear pore protein in Aspergillus nidulans using global proteomic analysis. Aysha H. Osmani, Hui-Lin Liu, Colin P. De Souza and Stephen A. Osmani. The Ohio State University, Columbus, OH, USA. (osmani.2@osu.edu)

We have previously reported the unexpected finding that in Aspergillus nidulans removal of all three fungal transmembrane nuclear pore complex (NPC) proteins (An-Ndc1, An-Pom152, and An-Pom34) does not cause lethality (Liu et al., 2009 MBC 20, 616-630). This suggests either that transmembrane proteins are not required for NPC function or that additional transmembrane NPC proteins remain to be discovered. To address this issue we have completed a global proteomic analysis of A. nidulans Nups (NPC proteins) using affinity purification and mass spectrometry analysis. This approach involved endogenously tagging >40 different proteins and completing LC/MS/MS analysis of >100 purified samples. The data set has allowed identification of numerous proteins that interact with NPCs including an orthologue of a new transmembrane Nup (Pom33) recently discovered in budding yeast (Chadrin et al., 2010 JCB 189, 795-811). An- Pom33 purified with An-Ndc1. Using GFP tagging and deletion analysis we have asked if An-Pom33 is a new transmembrane Nup in A. nidulans. As expected of a transmembrane Nup, An-Pom33-GFP locates to the nuclear envelope throughout interphase, concentrating at spindle pole bodies during mitosis in a manner similar to An-Ndc1. In addition, An-Pom33 is also present in the cytoplasm in an ER-like structure. Removal of An-Pom33 does not cause lethality but the quadruple transmembrane Nup mutant (lacking An-Ndc1, An-Pom152, An-Pom34, and An-Pom33) is inviable. Pairwise deletions revealed that lack of An-Pom33 and lack of An-Ndc1 is the only lethal combination. This study identifies An-Pom33 as a conserved transmembrane Nup that functions redundantly with An-Ndc1. (Supported by NIH grant GM042564)

241. Defining the essential location and function of Nup 2 in *Aspergillus nidulans.* Subbulakshmi Suresh, Sarine Markossian, Aysha H. Osmani and Stephen A. Osmani, Department of Molecular Genetics, The Ohio State University, USA-43210.

Nuclear Pore Complexes (NPCs) are macromolecular assemblies spanning the nuclear envelope and they are made up of 30 different proteins called Nucleoporins (Nups). In *Aspergillus nidulans*, the NPC undergoes partial disassembly during mitosis during which peripheral Nups disperse from the NPCs throughout the cell. However, Nup2 has a unique mitotic behavior as it translocates from NPCs onto chromatin specifically during mitosis. Nup2 is targeted to NPCs and chromatin by a newly identified Nup called NupA. We aim to define if the sole role of NupA is to target Nup2 to the NPC and chromatin by creating Nup2-NupA chimeras. We also aim to characterize whether the essential role of Nup2 is at NPCs during interphase, or on chromatin at mitosis, by artificially tethering Nup2 exclusively to NPCs or to chromatin. Also, since Nup2 is a mitotic phosphoprotein, we seek to define its phosphorylation sites. To answer the above aims, we have successfully created Nup2-NupA chimeras. We have also achieved artificial tethering of Nup2 to specific cellular locations and find that constitutive tethering of Nup2 to the NPC compromises cell viability. We also find that Nup2 is highly phosphorylated in response to activation of the spindle assembly checkpoint and have begun to map the phosphorylation sites using MS analysis of purified Nup2. In conclusion, our studies shed light on the mitotic functions of Nup2 on chromatin, regulated by mitotic kinases. (Supported by National Institutes of Health grant GM042564)

242. Cyclophilin D links programmed cell death and organismal aging in *Podospora anserina.* Andrea Hamann[1], Diana Brust[1], Bertram Daum[2], Christine Breunig[1], Werner Kühlbrandt[2], Heinz D. Osiewacz[1] [1]Johann Wolfgang Goethe University, Faculty for Biosciences & Cluster of Excellence Macromolecular Complexes, Institute of Molecular Biosciences; [2]Max Planck Institute of Biophysics, Department of Structural Biology; Frankfurt, Germany. e-mail: a.hamann@bio.uni-frankfurt.de

The ascomycete *Podospora anserina* is characterized by a well-established mitochondrial etiology of aging. Recently, we demonstrated the involvement of an apoptosis machinery in lifespan determination in this fungus. Here, we present another cell death determinant, the mitochondrial peptidyl prolyl-*cis*,*trans*-isomerase cyclophilin D (CYPD) which is known to be involved in opening of the mitochondrial permeability transition pore (mPTP). CYPD abundance increases during aging in mammalian tissues but also in the aging model organism *P. anserina*. Treatment of the wild-type with low concentrations of the cyclophilin inhibitor cyclosporin A (CSA) extends lifespan. Overexpression of *PaCypD* leads to reduced stress tolerance, pronounced mitochondrial dysfunction, accelerated aging and induction of cell death. CSA restores mitochondrial function and lifespan to that of the wild-type. In contrast, *PaCypD* deletion strains are not affected by CSA and show increased resistance against inducers of oxidative stress and cell death. Our data integrate programmed cell death (PCD) into a hierarchical network of pathways involved in the control of organismal aging and lifespan.

243. The BEM46-like protein from *N. crassa* is required for ascospore germination. K. Kollath-Leiss, F. Kempken Abt. Bot. Genetik und Molekularbiol., Bot. Institut und Bot. Garten, Olshausenstr. 40, 24098 Kiel, Germany; fkempken@bot.uni-kiel.de

The bud emergence (Bem) 46 proteins are members of the alpha/beta-hydrolase super family. The real function of these evolutionarily conserved proteins is still unknown (1). We used the model organism *Neurospora crassa* in conjunction with bem46 RNAi, over expression vectors and repeat induced point mutation to understand the cellular role of Bem46. While vegetative hyphae, perithecia and ascospores developed normally, hyphae germinating from ascospores showed a loss-of-polarity phenotype that indicates a role of Bem46 in maintaining cell type-specific polarity. We also investigated the subcellular localisation of Bem46 in *N. crassa*, and found that the protein is localized at or close to the plasma membrane and is targeted to the perinuclear endoplasmatic reticulum (2). The use of Lifeact-TagRFP and Bem46-eGFP in heterokaryons indicated that the Bem46 protein is not colocalized with actin. Likewise the use of TexasRedTM, which stains lipid rafts showed no co-localization with Bem46-eGFP. To identify interacting proteins a yeast two-hybrid approach was undertaken. We identified only one interacting protein, the anthranylate synthase component II (3). Currently in vivo interaction of both proteins is analyzed by employing BiFC assays. (1) Galperin MY et al. 2010 Trends Biotechnol 28:398-406 (2) Mercker M et al. 2009 Curr Genet 55:151-161 (3) Margaret S. Walker et al. 1986 J Biol Chem. 5;261(34):16073-7

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

244. Analysis of the phocein homologue SmMOB3 from the filamentous ascomycete *Sordaria macrospora.* Yasmine Bernhards and Stefanie Pöggeler Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Georg-August University, Grisebachstr. 8, D-37077 Göttingen, Germany, e-mail:spoegge@gwdg.de

Members of the striatin family and their highly conserved interacting protein phocein/Mob3 are key components in the regulation of cell differentiation in multicellular eukaryotes. The striatin homologue PRO11 of the filamentous ascomycete *Sordaria macrospora* has a crucial role in fruiting body development. Here, we functionally characterized the phocein/Mob3 orthologue SmMOB3 of *S. macrospora*. We isolated the gene and showed that both, *pro11* and *Smmob3* are expressed during early and late developmental stages. Deletion of *Smmob3* resulted in a sexually sterile strain, similar to the previously characterized pro11 mutant. Fusion assays revealed that DeltaSmmob3 was unable to undergo self fusion and fusion with the pro11 strain. The essential function of the SmMOB3 N-terminus containing the conserved mob domain was demonstrated by complementation analysis of the sterile *S. macrospora* DeltaSmmob3 strain. Downregulation of either *pro11* in DeltaSmmob3, or *Smmob3* in pro11 mutants by means of RNA interference (RNAi) resulted in synthetic sexual defects, demonstrating the importance of a putative PRO11/SmMOB3 complex in fruiting body development.

245. Spatial regulation of the spindle assembly checkpoint in *Aspergillus nidulans*. H. Edgerton-Morgan[1,2], T. Nayak[1], and B.R. Oakley[2] [1]Molecular Genetics, The Ohio State University, Columbus, OH. [2]Molecular Biosciences, University of Kansas, Lawrence, KS

The *Aspergillus nidulans* gamma-tubulin mutation *mipA*D159 causes a nuclear autonomous failure of inactivation of the anaphase promoting complex/cyclosome (APC/C) removing affected nuclei from the cell cycle (Nayak et al., 2010, J. Cell Biol. 190:317-330). This raised the question as to why the APC/C was not inactivated in mitosis in those nuclei by the spindle assembly checkpoint (SAC). *MipA*D159 causes additional defects in mitotic and cell cycle regulation without inhibiting microtubule nucleation (Prigozhina et al., 2004, Mol. Biol. Cell, 15:1374-1386) suggesting that gamma-tubulin may have additional roles in mitotic regulation. To determine if *mipA*D159 affects the SAC, we have tagged and imaged *A. nidulans* homologs of four components of the SAC: Mad2, Mps1, Bub3, and BubR1. The four proteins were spatially separated in interphase until they came together at mitotic onset forming the mitotic checkpoint complex. This implies that SAC activity is controlled in part by regulation of the locations of its components. In strains carrying *mipA*D159, BubR1 failed to accumulate at the SPB/K in some nuclei. BubR1 has two APC/C recognition sequences and is likely destroyed in these nuclei by a constitutively active APC/C, preventing activation of the SAC. Mad2, Bub3 and Mps1 localized to the SPB/K normally when BubR1 was absent and they, thus, do not depend on BubR1 for SPB/K localization. We have found that, unlike BubR1, Bub3 and Mad2, Mps1 is essential for viability. This indicates that Mps1 has an essential function independent of the SAC. Supported by NIH grant GM031837.

246. The *snxA* gene of *Aspergillus nidulans* encodes a homolog of yeast Hrb1. S.L. McGuire Anglin[1], T. Banta[1], C. Coile[1], C. Dixit[1], S. Eastlack[1], A. Giang[1], J. Kobie[2], M. Nguyen[1], K. Shingler[2], A. Orzechowski[2], and S. James[2]. [1]Millsaps College, Jackson, MS. [2]Gettysburg College, Gettysburg, PA. mcguisl@millsaps.edu

The *snxA1* cold-sensitive mutation of *A. nidulans* was originally identified as an extragenic suppressor of the *nimX2*^{F223L} heat sensitive mutation. Analysis of double mutants showed that *snxA1* suppresses all three heat sensitive *nimX*^{CDC2} mutations as well as mutations in *nimE*^{CYCLINB} and *nimT*^{CDC25}; that *snxA1* has a synthetic phenotype in combination with a deleted *ankA*^{WEE1}; and that *snxA1* is not a general cell cycle suppressor. Western blot data suggest that the levels of both NIMX and CYCLINB are not affected by the *snxA1* mutation. We have recently cloned snxA and found that it encodes a homolog of *Saccharomyces cerevisiae* Hrb1, a protein involved in mRNA transcription and export. SNXA is nonessential; however, deletion of *snxA* results in an extremely cold-sensitive phenotype similar to but more extreme than the *snxA1* mutation; deletion also suppresses *nimX*^{CDC2} mutations. Overexpression of wild-type SNXA complements *snxA1*. These data suggest that nonmutant SNXA functions to restrain NIMX/CYCLINB activity. GFP-labelled SNXA localizes to the nucleus in 87% of germlings when grown at 29°C; 13% of germlings have no detectable SNXA. Studies are currently underway to determine if SNXA localization correlates with CYCLINB localization. Funding provided by NIH NCRR P20RR016476 and NIH2R15GM055885

247. Does RAS-1 regulate adelylate cyclase activity? Wilhelm Hansberg, Sammy Gutiérrez, Pablo Rangel. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, UNAM, México, whansberg@ifc.unam.mx

In *Neuropspora crassa*, conidiation is started when an aerated liquid culture is filtered and the resulting mycelial mat is exposed to air. Three morphogenetic transitions take place: hyphae adhesion, aerial hyphae growth and conidia development. Each transition is started by an unstable hyperoxidant state and results in growth arrest, autophagy, antioxidant response and a dioxygen insulation process. These responses stabilize the system and, once stable, growth can start again. In a solid medium the *bd* mutant exhibits a conidiation band every 22 h. *bd* has a Thr79Ile substitution in *ras-1*. Compared to Wt, *ras-1*^{bd} has increased ROS formation during conidiation resulting in increased aerial mycelium growth and increased submerged conidiation. Our hypothesis is that RAS-1 acts as a switch between growth and conidiation in *N. crassa*. Only three proteins have a predicted RAS association domain: NRC 1, the STE50p orthologue and adenylate cyclase (AC). A Δ*cr- 1* mutant strain decreases grow of vegetative and aerial hyphae and increases conidia formation. Upon exposure to air, cAMP levels in a mycelial mat follow a similar pattern to protein oxidation, loss of NAD(P)(H)-reducing power and glutathione oxidation. cAMP levels decrease during the hyperoxidant state, both at the start of hyphal adhesion and of aerial hyphae formation, and recover thereafter. AC and the low affinity phosphodiesterase (NCU00237) activity regulation explained cAMP decrease. However, during conidia formation, cAMP decrease was due to regulation of AC and the high affinity phosphodiesterase (NCU00478).

248. Shedding light on long-distance movement of protein during organogenesis of the fruiting body in *Agaricus bisporus*. Benjamin Woolston[1], Carl Schlagnhaufer[2], Jack Wilkinson[3], Jeffrey Larsen[1], Zhixin Shi[3], Kimberly Mayer[3], Donald Walters[3], Wayne Curtis[1], and Peter Romaine[2,3]. Departments of [1]Chemical Engineering and [2]Plant Pathology, The Pennsylvania State University, University Park, Pennsylvania USA. [3]Agarigen, Inc., Research Triangle Park, North Carolina USA.

Commercial cultivation of *Agaricus bisporus* (button mushroom) is carried out in a bi-layered system consisting of a compost bed and a peat overlay, each of which is mixed with an *Agaricus* mycelial inoculant. We discovered that seeding the lower compost layer with a transgenic inoculant carrying a beta-glucuronidase (*GUS*) reporter gene and the upper peat layer with a wild-type inoculant resulted in the formation of fruiting bodies exhibiting GUS activity while lacking the cognate transgene. Results of PCR and RT-PCR analyses were consistent with the shuttling of the GUS protein, rather than its cognate mRNA, from the mycelium colonizing the compost layer into the fruiting body that developed from wild-type mycelium colonizing the peat layer. Moreover, the genotype of the fruiting body was determined solely by the genotype of the *Agaricus* inoculant used in the upper peat substrate, irrespective of the inoculant in the underlying compost. A double-inoculant strategy, in which the two substrate layers were inoculated with individual transgenic lines with the *GUS* gene under the control of different tissue-preferred promoters, provided up to a six- fold increase in GUS activity relative to that obtained with a single transgenic line seeded throughout the growing medium. Our findings provide insight into a previously unreported phenomenon of long-distance protein mobilization in a fungus and offer a novel strategy to increase recombinant protein production in *A. bisporus*. This research was supported by funding from the DARPA – Accelerated Manufacture of Pharmaceuticals Program and The Pennsylvania State University – J. B. Swayne Chair in Spawn Science.

249. Withdrawn

250. Identification of a microtubule associating protein that interacts with nuclear pore complex proteins during mitosis. Nandini Shukla, Aysha H. Osmani, Stephen A. Osmani. The Ohio State University, Columbus, OH, USA. (shukla.25@buckeyemail.osu.edu)

Aspergillus nidulans exhibits partially open mitosis wherein the nuclear pore complexes (NPCs) undergo partial disassembly similar to the initial stages of open mitosis of higher eukaryotes. It is currently not known how the partial disassembly and re-assembly of NPCs is carried out or regulated. One potential mechanism is the existence of NPC disassembly factors that would bind preferentially to NPC proteins (Nups) during mitosis to promote their disassembly from the core structure. The current work describes efforts to identify such proteins using affinity purification - MS analyses of Nups from interphase and mitotic cells. An interesting novel protein, ANID_03906, identified by this approach preferentially co-purifies with mitotic Gle1, Nup133, and Mad1. Endogenous GFP tagging revealed that ANID_03906, locates to cytoplasmic microtubules during interphase. It appears to both coat microtubules and form mobile foci that move along microtubules. Drug treatment to depolymerize microtubules dramatically modifies the location of ANID_03906 which locates to immobile aggregates without microtubule function. These data suggest this previously unstudied protein might play a role during interphase involving microtubules and at mitosis involving specific Nups. Future work aims at understanding the interactions of ANID_03906 with Nups and components of the cytoskeleton during cell cycle progression. (Supported by NIH grant GM042564)

251. Macroautophagy-mediated Degradation of Whole Nuclei in the Filamentous Fungus *Aspergillus oryzae*. Jun-ya Shoji, Takashi Kikuma, Manabu Arioka, and Katsuhiko Kitamoto Department of Biotechnology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan email; junya@fungalcell.org

Filamentous fungi consist of continuum of multinucleate cells called hyphae, and proliferate by means of hyphal tip growth. Accordingly, research interest has been focusing on hyphal tip cells, but little is known about basal cells in colony interior that do not directly contribute to proliferation. Here, we show that autophagy mediates degradation of basal cell components in the filamentous fungus *Aspergillus oryzae*. In basal cells, enhanced green fluorescent protein (EGFP)-labeled peroxisomes, mitochondria, and even nuclei were taken up into vacuoles in an autophagy-dependent manner. During this process, crescents of autophagosome precursors matured into ring-like autophagosomes to encircle apparently whole nuclei. The ring-like autophagosomes then disappeared, followed by dispersal of the nuclear material throughout the vacuoles, suggesting the autophagy-mediated degradation of whole nuclei. We also demonstrated that colony growth in a nutrient-depleted medium was significantly inhibited in the absence of functional autophagy. This is a first report describing autophagy-mediated degradation of whole nuclei, as well as suggesting a novel strategy of filamentous fungi to degrade components of existing hyphae for use as nutrients to support mycelial growth in order to counteract starvation.

252. Septum-directed secretion in the filamentous fungus *Aspergillus oryzae*. Jun-ya Shoji, Yugo Hayakawa, Eri Ishikawa, and Katsuhiko Kitamoto Department of Biotechnology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan email; junya@fungalcell.org

Although it is generally believed that exocytosis in fungal cells exclusively takes place at hyphal tips, there also seems a line of circumstantial evidence suggesting the occurrence of exocytosis at other sites of cells, such as septa. To investigate whether exocytosis takes place at fungal septa, we monitored dynamics of EGFP-fused AmyB (AmyB-EGFP), the representative secretory enzyme of the filamentous fungus *Aspergillus oryzae*. We found that AmyB-EGFP accumulates in Spitzenkörper at hyphal tips as well as septal periplasm between the plasma membrane and cell walls. The septal accumulation of AmyB-EGFP was a rapid process, and required microtubules but not F-actin. Thus, this process is independent of exocytosis at hyphal tips that requires both microtubules and F-actin. In addition, fluorescence recovery after photobleaching (FRAP) analysis of EGFP-fused AoSnc1 revealed that secretory vesicles constitutively fuse with septal plasma membrane. These results demonstrated that exocytosis takes place at septa in addition to hyphal tips. Analysis of two plasma membrane transporters, AoUapC and AoGap1, revealed that they preferentially accumulate at septa and lateral plasma membrane with no clear accumulation at apical Spitzenkörper, suggesting that non-tip directed exocytosis is important for delivery of these proteins.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

253. Aspergillus nidulans GputA (galactose-1-phosphate uridylyltransferase) is not essential for galactose metabolism, but is required for wild type conidiation. Md Kausar Alam and Susan Kaminskyj, Dept Biology, Univ Saskatchewan, Canada. mda514@mail.usask.ca

Saccharomyces galactose-1-phosphate uridylyltransferase (GAL7) mediates transfer of UDP between galactose and glucose and their respective sugar-1-phosphate conjugates, leading to glycolysis or to wall glycan synthesis. Aspergillus nidulans ANID_06182 has 50% amino acid sequence identity with GAL7 and was annotated as a galactose-1-phosphate uridylyltransferase. We named it GputA. Although GAL7 is essential, a confirmed gputA deletion strain, AKA1 grew and conidiated on minimal medium containing glucose or galactose as sole carbon sources, and on peptide-only Difco Nutrient Broth. AKA1 conidiation was reduced compared to a near-isogenic gputA+ strain, AAE1 on complete medium containing glucose or galactose (CM-Glu or CM-Gal) as sole carbohydrate sources. Scanning electron microscopy showed this was due to failure at the vesicle-metula transition on CM-Glu vs reduced spore production from morphologically normal phialides on CM-Gal. AKA1 spore germination was 78% on CM-Glu but 1% on CM Gal. GputA-GFP fluorescence was cytoplasmic, was more intense in spores than hyphae, and was significantly brighter for cells grown on CM-Gal. Using qRT-PCR, we found that gputA expression was enhanced ten-fold by growth on CM-Gal compared to CM-Glu. These findings suggest that GputA function is distinct from Saccharomyces GAL7, and is consistent with A. nidulans hexose metabolism complexity. AKA1 defects appear to relate to cell polarity establishment but not polarity maintenance.

254. Spatial regulation of cellular morphogenesis in Aspergillus nidulans by components of Cdc42/Rac GTPase modules. Brad Downs, Haoyu Si, and Steven D. Harris, Dept. of Plant Pathology and Center for Plant Science Innovation, University of Nebraska, Lincoln, USA 68588-0660, Bdowns@huskers.unl.edu .

In filamentous fungi such as A. nidulans, polarized growth occurs via localized cell wall deposition and cell surface expansion at discrete sites such as hyphal tips. In mature hyphae, additional growth sites are generated by the formation of lateral branches from sub-apical cells. Conversely, during conidiation, branch formation appears to be suppressed in developing conidiophores. During prior studies of the Cdc42 and RacA GTPases in A. nidulans, we found that Cdc42 is required for hyphal branching. Here, as part of an effort to further understand the regulation of these GTPases, we describe the functional characterization of the predicted Cdc42/Rac GAP RgaA (homologue of yeast Rga1) and the candidate effector PakB (homologue of yeast Cla4). Notably, we find that the loss of each protein results in altered patterns of hyphal branching. More intriguingly, RgaA and PakB both contribute to suppression of branching during conidiation, as the loss of either leads to the formation of conidiophores with abnormally branched stalks, vesicles, and metulae. In the case of RgaA, double mutant analysis suggests that this phenotype is caused by hyperactive Cdc42. We also find that PakB localizes to a crescent at hyphal tips, as well as to growth sites in conidiophores. Collectively, our genetic observations implicate RgaA and PakB in the suppression of branch formation during hyphal growth and development.

255. Different regulation of five beta-1,3-glucanosyltransferase genes in *Neurospora crassa*. Masayuki Kamei, Kazuhiro Yamashita, Masakazu Takahashi, Akihiko Ichiishi and Makoto Fujimura, Faculty of Life Sciences, Toyo University, Gunma, Japan

The Gel/Gas/Phr proteins belong to glycoside hydrolase family 72 are new family of beta-1,3-glucanosyltransferase. These GPI-anchored proteins play an important role in fungal cell wall biosynthesis. *N. crassa* has five putative beta-1,3-glucanosyltransferase genes, *gel-1*, *gel-2*, *gel-3*, *gel-4*, and *gel-5*, in its genome. Among them, the *gel-3* gene is constitutively expressed at most high level during conidial germination and hyphal growth, whereas basal expression of *gel-1* gene is lowest. The *gel-3* deletion mutant displayed slow growth, while other *gel* gene disruptants showed the normal growth. This suggested that the GEL-3, an ortholog of *Fusarium oxysporum* GAS1 and *Aspergillus nidulans* GEL4, is most important beta-1,3- glucanosyltransferase in normal growth condition. Although any *gel* gene disruption did not affect the pH sensitivity, all of *gel* disruptants were more resistant to cell wall degradation enzymes than the wild-type. Micafungin, a beta- 1,3-glucan synthase inhibitor, upregulated *gel-4* expression at almost 4-fold in the wild-type. In contrast, fludioxonil, an activator of OS-2 MAP kinase, strongly induced *gel-1* gene, more than 50-fold upregulation, in the wild-type. Its induction was almost abolished in the *os-2* disruptant. These suggested that *gel-1*, *gel-3*, and *gel- 4* genes are differently controlled probably to maintain the cell wall integrity.

256. Scavenging of reactive oxygen species (ROS) as part of a hierarchical network of mitochondrial pathways involved in aging and lifespan control. Heinz D. Osiewacz[1], Andrea Hamann[1], Edda Klipp[2], Axel Kowald[2], Sandra Zintel[1]. [1]Johann Wolfgang Goethe University, Faculty of Biosciences and Cluster of Excellence Macromolecular Complexes, Frankfurt, Germany. [2]Humboldt University, Institute for Biology, Theoretical Biophysics, Berlin, Germany. E-Mail: Osiewacz@bio.uni-frankfurt.de

Biological aging is controlled by a complex mitochondrial network of interacting molecular pathways. Here we report the effect of a specific genetic manipulation of different components of the ROS scavenging system of the fungal aging model *Podospora anserina*. Unexpectedly, we found that the deletion of the gene coding for the mitochondrial manganese superoxide dismutase (PaSOD3) did not significantly affect the lifespan while over-expression let to severe impairments (e.g., growth rate, sensitivity against exogenous stressors) including a reduction in lifespan. Most strikingly, the up-regulation of only a single gene had a strong impact on the abundance of a number of proteins from different molecular pathways (e.g., ROS scavenging, proteolysis, heat-shock response) demonstrating the need for careful and systematic analyses of the effect of specific genetic manipulations. Such an analysis, which may utilize approaches of Systems Biology, is important to elucidate the impact and the interactions of individual pathways which in the past have been identified to contribute to biological aging.

257. The GPD1-GPP1-independent glycerol biosynthesis pathway is essential for osmotic adaptation in filamentous ascomycetous fungi. Kosuke Izumitsu, Hajime Kobayashi, Akira Yoshimi, Yoshimoto Saitoh, Atsushi Morita, Chihiro Tanaka. Kyoto University, Graduate School of Agriculture, Kyoto, Japan.

Recent studies have suggested that glycerol biosynthesis pathways differ between filamentous ascomycetous fungi and the budding yeast *Saccharomyces cerevisiae*. Here, we characterized genes associated with glycerol biosynthesis in the filamentous fungus *Cochliobolus heterostrophus* and *Botrytis cinerea*. Our genealogic study revealed that *Gld1* and *Cut1* genes, which encode glycerol dehydrogenase and HAD-type phosphatase, respectively, were specific to filamentous ascomycetes and absent in the budding yeast. These two genes were arranged in a head-to- head configuration, which is highly conserved among various filamentous ascomycetes, implying a close functional relationship between Gld1p and Cut1p. While under osmotic stress, *Cut1* and *Gld1*, but not *Gpd1* and *Gpp1*, is strongly induced via HOG1-type MAPK. Cut1p and Hog1p, but not Gpd1p, were essential for accumulating intracellular glycerol under the osmotic shock in both fungal species. These results indicate that unlike budding yeast, the CUT1 pathway is essential for the osmotic induction of glycerol. Moreover, the double-mutant strain *Gpd1/Cut1* of *C. heterostrophus* did not grow on media without glycerol, while single-mutant strains grew normally. These results indicate that the GPD1-GPP1 and CUT1 pathways redundantly regulate basal levels of glycerol biosynthesis, which is essential for vegetative growth.

258. Characterization of the Rab GTPase gene *BcSec4* in *Botrytis cinerea.* Chihiro Tanaka, Syunichi Kimura, Hajime Kobayashi, Yoshimoto Saitoh, Atsuhi Morita, Kosuke Izumitsu. Kyoto University, Graduate School of Agriculture, Kyoto, Japan.

Botrytis cinerea, the causal agent of grey mold desease, is a plant pathogen with a very wide host range (over 200 species), resulting in heavy damage to various plants all over the world. Interestingly, Mepanipyrim, one of the most effective fungicides for grey mold disease, have been revealed to be a protein secretion inhibitor. Then, we presumed that the secretion systems of proteins, including cell wall degrading enzymes, have important roles for pathogenicity of *B. cinerea*. Therefore, we focus on *Sec4* gene, known as a member of Rab GTPases, which plays a important role in vesicular transport from trans-Golgi network to cellular membrane. We cloned *BcSec4*, homologous to yeast *Sec4* gene, in *B. cinerea*. We obtained 2 null-mutants of *BcSec4* by homologous recombination. *BcSec4* mutants had the less ability to conidiate on a medium at 25 ºC. *BcSec4* mutants tended to form overmuch aerial mycelia and hardly reach the edge of petri dish, even a few months after inoculation. At cold condition (15 ºC), *BcSec4* mutants produced abundant aerial mycelia, but sclerotia, while WT produced abundant sclerotia, but not conidia on the medium. The pathogenicity of *BcSec4* mutants on tomato leaves was also investigated. *BcSec4* mutants formed smaller lesions than WT. We therefore conclude that *BcSec4* is associated with conidiation, sclerotinia formation, and pathogenicity.

259. On the role of a new member of the CDK9 kinase family in *A. nidulans.* Claudia Kempf, Friederike Bathe and Reinhard Fischer Karlsruhe Institute of Technology , Dept. of Microbiology, Karlsruhe, Germany Claudia.Kempf@KIT.edu

Cyclin dependent kinases (CDKs) are a large group of protein kinases which are regulated by association with cyclins. Members of the Cdk9 family have been described from yeast to human and are known to be part of the basal transcription elongation machinery. Their regulatory subunits are different cyclins (cyclin T1, T2a, T2b and K), which do not oscillate during the cell-cycle. In *A. nidulans* the cyclin PclA has been characterized as a cyclin involved in development. PclA interacts with the main regulator of the cell cycle, NimX and may help to adjust the cell cycle during asexual sporulation (1). In a targeted approach it was found that PclA interacts with another kinase, a Cdk9 family member (PtkA) (2). Deletion of the *ptkA* gene is lethal and the mutant arrests in a short germling state. PtkA localizes to nuclei during interphase. PtkA does also interact with a cyclin T (PchA) as it does in other organsims, suggesting a conserved role in transcription regulation. Performing Y2H screens with PtkA, we identified two more interaction partners, one protein kinase and surprisingly, another Pcl cyclin. These interactions occurred most interestingly only in metulae and phialides and are thus restricted to asexual development. These results point to the possibility that the transcription elongation machinery is specifically modified during asexual development. (1) Schier et al. (2002), *FEBS Lett.* 523: 143-6 (2) Bathe et al. (2010), *Eukaryot Cell.* 9: 1901-12

260. Analysis of the specificity of the kinesin-3 motor UncA for detyrosinated microtubules in *Aspergillus nidulans.* Constanze Seidel, Nadine Zekert and Reinhard Fischer Karlsruhe Institute of Technology, Dept. of Microbiology, Karlsruhe, Germany constanze.seidel@kit.edu

Molecular motors are involved in many processes such as transport of vesicles, organelles and proteins. The *A. nidulans* motor UncA belongs to the kinesin-3 family and transports vesicles required for hyphal extension. UncA- dependent vesicle movement occurred preferentially along MTs probably composed of detyrosinated alpha-tubulin, a subpopulation of the tubulin cytoskeleton (1). To understand how UncA is able to distinguish between “normal” (tyrosinated) and detyrosinated MTs, deletion analyses revealed a region in the tail between amino acid 1316 and 1402. A non-targeted Y2H approach was used to identify interaction partners of this region, which are most likely involved in recognition of MT subpopulations. Two candidates appeared to be associated with vesicles and currently split-YFP assays with UncA are performed to confirm these interactions. To investigate mechanisms that regulate specificity, transport and cargo recognition of UncA, we are characterizing the role of Rab-3 and their specific DENN domain orthologues, which interact with the SNARE complex (2). Deletion of one DENN protein caused a strong reduction of growth and sporulation. GFP-DENN fusion proteins localized to fast moving spots and are associated with the cytoplasmic membrane; suggesting a role in tethering UncA to endocytotic vesicles. (1) Zekert N. & Fischer R. (2009) *Mol Biol Cell* 20(2):673-84 (2) Niwa S. et al. (2008) *Nat Cell Biol* 10(11):1269-79

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

261. Functional analysis of SPFH domain-containing proteins, Flotillin and Stomatin, in *Aspergillus nidulans.* Norio Takeshita, Reinhard Fischer. Karlsruhe Institute of Technology, Karlsruhe, Germany, norio.takeshita@kit.edu

Polarized growth of filamentous fungi depends on the microtubule and the actin cytoskeleton. Apical membrane- associated landmark proteins, so-called "cell end markers" link the two cytoskeletons. Our latest results indicate that apical sterol-rich membrane domains (SRDs) play important roles in polarized growth and localization of cell end markers. The roles and formation mechanism of SRDs remain almost unknown. To analyze the functional roles of SRDs, we are investigating the mechanism of SRD (or raft cluster) formation and maintenance. There are numerous studies on raft formation in different organisms. Flotillin/reggie proteins for instance were discovered in neurons and are known to form plasma membrane domains. The flotillin/reggie protein and a related microdomain scaffolding protein, stomatin, are conserved in filamentous fungi but have not yet been characterized. We have started the investigation of their functions by gene deletion and GFP-tagging. It was revealed that the flotillin/reggie protein FloA-GFP accumulated at hyphal tips. Deletion of *floA* caused a reduction of the growth rate and often irregular shaped hyphae. Moreover, the stomatin related protein StoA-GFP localized at young branch tips and at the subapical cortex in mature hyphal tips. Deletion strains of *stoA* also showed smaller colonies than wild-type and exhibited irregular hyphae and increased branching. The localization of SRDs, cell end markers, and actin etc. are being analyzed in the mutants.

262. Nuclear dynamics during cell cycle and hyphal fusion of *Fusarium oxysporum.* C. Ruiz-Roldán[1*], M. Köhli[2], M. I. G. Roncero[1], P. Philippsen[2], A. Di Pietro[1] and E. A. Espeso[3] [1]University of Cordoba, Spain; [2]University of Basel, Switzerland; [3]CIB-CSIC, Madrid, Spain [*]E-mail: ge2rurom@uco.es

Similar to other fungal pathogens, the early stages of interaction between *Fusarium oxysporum* and its host are crucial for the outcome of infection, including spore germination, adhesion to the host surface, establishment of hyphal networks through vegetative hyphal fusion (VHF) and penetration of the host. The aim of this study was to explore nuclear dynamics during the different developmental stages of *F. oxysporum.* Fusion PCR-mediated gene targeting was used to C-terminally label histone H1 with either GFP or ChFP, allowing us to perform, for the first time, live-cell analysis of nuclear dynamics in this species. Our study revealed the presence of two distinct nuclear pedigrees of mitotic activity. Asexual conidiation follows the typical basopetal pattern of a stem cell lineage, while vegetative hyphal cells maintain a strictly acropetal pattern, defining *F. oxysporum* as a mononucleated mycelial organism. We provide evidence for a previously unreported cellular mechanism activated after vegetative fusion of two uninucleated cell compartments. The process consists of a nuclear division, followed by migration of an "invading nucleus" through the anastomosis bridge and subsequent degradation of the resident nucleus. These results suggest that this fungus displays a highly elaborate mechanism for restoring nuclear numbers and maintaining cell integrity after VHF, raising questions regarding the origin and role of post-fusion nuclear degradation, the signals that trigger the process and about how two genetically identical nuclei sharing a common cytoplasm can undergo such distinct developmental programs. We are performing further studies addressing these questions that will help to unravel the intricacies of the VHF process in *F. oxysporum*.

263. The genetic basis of conidial pigmentation in *Aspergillus niger.* M. Arentshorst, T.R. Jørgensen, J. Park, A.M. van Welzen, G. Lamers, P.A. vanKuyk, R.A. Damveld and A.F.J. Ram. Institute of Biology Leiden, Leiden University, Molecular Microbiology and Biotechnology, Kluyver Centre for Genomics of Industrial Fermentation, Sylviusweg 72, 2333 BE, Leiden, The Netherlands.

A characteristic hallmark of *Aspergillus niger* is the formation of black conidiospores. To understand the genetic basis of black spore formation, we have identified four genes required for pigmentation by using a complementation approach. First, we characterized a newly isolated color mutant, *colA*, which lacked pigmentation resulting in white conidia. Pigmentation of the *colA* mutant was restored by a gene (An12g03950) which encodes the *A. niger* ortholog of the 4'-phosphopantetheinyl transferase protein (PptA). The loci giving rise to fawn, olive, and brown color phenotypes were identified by complementation. The fawn mutant was complemented by the polyketide synthase A protein (PksA, An09g05730), the *ovlA* mutant by An14g05350 (OlvA) and the *brnA* mutant by An14g05370 (BrnA), the respective homologs of *pksP/alb1, ayg1* and *abr1* in *A. fumigatus*. Targeted disruption of the four genes confirmed the complementation results. Epistasis was determined for *pksA, olvA* and *brnA* by constructing double mutants. This set of isogenic color mutants is a useful tool to do classical genetic analyses in *Aspergillus niger*.

264. The membrane mucin Msb2 controls invasive growth and plant infection in *Fusarium oxysporum* . Elena Perez-Nadales and Antonio Di Pietro, Department of Genetics, University of Cordoba, Spain.

Fungal pathogenicity on plants requires a conserved mitogen-activated protein kinase (MAPK) cascade homologous to the yeast filamentous growth pathway. How this signalling cascade is activated during infection remains poorly understood. In the soilborne vascular wilt fungus *Fusarium oxysporum* , the orthologous MAPK Fmk1 is essential for root penetration and pathogenicity on tomato plants. Here we show that Msb2, a highly glycosylated transmembrane protein is required for surface-induced phosphorylation of Fmk1 and contributes to a subset of Fmk1-controlled functions related to invasive growth and virulence. Mutants lacking Msb2 share characteristic phenotypes with D*fmk1* mutants including defects in cellophane invasion, penetration of the root surface and induction of vascular wilt symptoms on tomato plants. In contrast to the D*fmk1* strains, D*msb2* mutants were hypersensitive to cell wall targeting compounds, a phenotype that was exacerbated in a D*msb2*D*fmk1* double mutant. These results suggest that the membrane mucin Msb2 promotes invasive growth and plant infection upstream of Fmk1 while contributing to cell integrity through a distinct pathway.

265. A potential functional relationship between phosphorylation by mitotic kinases and protein methylation by the Set1 complex in *Aspergillus nidulans.* Meera Govindaraghavan[1], Sarah Lea McGuire[2] and Stephen A. Osmani[1] [1]Department of Molecular Genetics, The Ohio State University, Columbus, OH, [2]Department of Biology, Millsaps College, Jackson, MS, govindaraghvan.1@buckeyemail.osu.edu, osmani.2@osu.edu

The G2-M transition is regulated by the activity of two mitotic kinases, NIMA and NIMX, in *Aspergillus nidulans*. To gain further insight into the mechanism of NIMA function, a synthetic lethal screen was carried out utilizing the deletion of the non-essential *nimA* orthologue, *KIN3*, in Saccharomyces cerevisiae. This screen revealed a set of 11 genes involved in different cellular processes. By deletion analysis, four of these synthetic genetic interactions were found to be conserved in *A. nidulans*, one of which is between *nimA7*^{ts} and the deletion of *An- swd1*, the ortholog of a subunit of the Set1 methyl transferase complex. Moreover, the synthetic lethal interaction between *An-swd1* and a cell cycle mutant with reduced NIMX function (*nimT23*^{ts}) suggests that lack of *An-swd1* function in combination with defects in G2-M transition is highly deleterious. These genetic interactions result from loss of protein methyl transferase activity of the Set1 complex, since the deletion of *An- set1*, which encodes the catalytic protein, also exhibits genetic interaction with *nimA7*^{ts} and *nimT23*^{ts}. Interestingly, the deletion of *An-swd1* also modifies the *nimA7* and *nimT23* phenotypes at their fully restrictive temperature, causing a drastic growth defect. Furthermore, a proportion of *nimA7+ An-swd1* cells are uninucleated yet undergo septation, a phenotype never observed in either single mutant. Collectively these results indicate an important functional relationship exists between mitotic protein phosphorylation and protein methylation.

266. DenA is a deneddylating protein involved in *A. nidulans* development. Martin Christmann, Rebekka Harting, Özgür Bayram, Gerhard H. Braus, Institute for Microbiology and Genetics, Georg August University Göttingen, D-37077 Göttingen, Germany (Germany), mchrist@gwdg.de

Deneddylation is the removal of the ubiquitin (Ub)-like protein Nedd8 from cullins. Cullins are subunits of cullin-RING Ub ligases (CRL) which are controlled in their activity and assembly/reassembly by neddylation and deneddylation, respectively. The most important eukaryotic deneddylases are the COP9 signalosome (CSN) and the deneddylating enzyme 1 (DEN1). Mammalian Den1 has two functions: an isopeptidase activity removing Nedd8 from cullins and other proteins and an additional linear peptidase activity processing Nedd8 from a precursor protein. Filamentous fungi possess an eight subunit COP9 signalosome (CSN) which is reminiscent to the corresponding plant and vertebrate complex (Busch et al. 2007, Braus et al., 2010). *Aspergillus nidulans* requires CSN function to trigger development, the appropriate response of the fungus towards light, and for a coordinated secondary metabolism (Nahlik et al., 2010). We show here the characzterization of the fungal Den1 ortholog DenA. The *denA* gene encodes a cysteine protease deneddylating enzyme. DenA is required for light control and the asexual fungal development whereas CSN is required for the sexual cycle. Processed Nedd8 is unable to rescue conidia formation suggesting that the lack of the DenA deneddylase isopeptidase activity is responsible for the defect. Yeast two hybrid studies suggest a physical interaction between DenA and CSN which has to be further evaluated. Busch S, Schwier EU, Nahlik K, Bayram Ö, Draht OW, Helmstaedt K, Krappmann S, Valerius O, Lipscomb WN, Braus GH (2007), PNAS, USA. 104, 8125-8130. Braus GH, Irniger S, Bayram Ö (2010), Curr. Opin. Microbiol. 13, 1-5. Nahlik K, Dumkow M, Bayram Ö, Helmstaedt K, Busch S, Valerius O, Gerke J, Hoppert M, Schwier E, Opitz L, Westermann M, Grond S, Feussner K, Goebel C, Kaever A, Meinecke P, Feussner I, Braus GH (2010), Mol. Microb. 78, 962-979.

267. Specificity determinants of GTPase recognition by RhoGEFs in *Ustilago maydis.* Britta Tillmann, Sonja Helene Frieser, Kay Oliver Schink and Michael Bölker FB Biologie, Universität Marburg, Karl-von-Frisch-Str.8, 35032 Marburg, Germany, email: britta-tillmann@gmx.de

Small GTPases of the Rho family act as molecular switches and are involved in the regulation of many important cellular processes. They are activated by specific guanine nucleotide exchange factors (RhoGEFs). In their active GTP bound state RhoGTPases interact with downstream effectors and trigger cellular events. The number of both RhoGEFs and effectors exceeds the number of GTPases, which raises the question how signalling specificity is achieved. In recent years the importance of RhoGEF specificity became more and more evident, as these upstream activators are often connected to their downstream effectors by scaffolding proteins. We analysed all *U. maydis* Cdc42-specific RhoGEFs (Don1, Its1 and Hot1) for their role in Cdc42 signalling both *in vivo* and *in vitro*. Interestingly, the GTPase recognition mechanism differs between Hot1 and the other two RhoGEFs. While amino acid at position 56 of Cdc42 is critical for GEF recognition of Don1 and Its1, Hot1 uses a different amino acid to discriminate between Cdc42 and Rac1. We identified additional amino acids which are important for GTPase recognition by Hot1. In future we will try to find out whether orthologs of Hot1 in other organisms use a similar mechanism to discriminate between GTPases.

268. Gold nanoparticles in Aspergillus nidulans hyphae: can we study real-time physiology? Susan Kaminskyj (a), Martin Prusinkiewicz (a), Fatemeh Faraz-Khorasani (b), Merrill Isenor (b), Kathleen Gough (b) a) Dept Biology, Univ Saskatchewan, Canada; b) Dept Chemistry, Univ Manitoba, Canada. Susan.Kaminskyj@usask.ca

High spatial resolution methods to analyze biochemical composition of individual hyphae can assess cell physiology during growth in optimal or stressed conditions. Whole colony methods like GC-MS cannot capture all the details of physiology and organism-environment interaction. In addition to Fourier transform infrared (FTIR) spectromicroscopy, surface-enhanced Raman spectroscopy (SERS) can provide biochemical characterization of components that are in contact with gold that has been nano-patterned (Klarite substrate) or has formed nano-particles (AuNPs). SERS can potentially be used to examine biochemical processes in living cells. We have grown AuNPs within and on the surface of Aspergillus nidulans hyphae, and documented their distribution and composition using transmission electron microscopy and scanning transmission x-ray microscopy. Most AuNPs were associated with hyphal walls, both in the cytoplasm and on the wall surface. AuNPs grown in cultures treated for 2 h with 1 mM Au3+ appeared to be optimal for generating SERS activity. The AuNP spectra were more complex than most SERS spectra from A. nidulans hyphae grown on a Klarite substrate. Interpreting SERS spectra will be challenging, and will require validation for the diversity of molecules present on the wall and in the peripheral cytoplasm. To date we have proof in principle that it will be possible to generate SERS spectra in living hyphae.

269. Synthetic reduction of Cdc42/Rac1 GTPase signalling complexity in *Ustilago maydis.* Sonja Helene Frieser, Kay Oliver Schink, Britta Tillmann and Michael Bölker FB Biologie, Universität Marburg, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany; email: Sonja.Frieser@web.de

Small GTP binding proteins of the Rho family play important roles as molecular switches that trigger many cellular functions. Cdc42 and Rac1 are highly conserved Rho-GTPases which regulate cytokinesis and polarized growth in *U. maydis*. Neither Cdc42 nor Rac1 is essential but depletion of both results in lethality. We have generated a synthetic haploid *U. maydis* strain, which contains Cdc42 as sole GTPase of the Rac1/Cdc42 pair and displays no obvious growth defect or any other morphological phenotype. This synthetic reduction of GTPase signalling complexity was reached by engineering the activator Cdc24 from a Rac1-specific into a Cdc42-specific GEF. In this synthetic strain Cdc42 is able to fulfill all biological functions of Rac1 although all downstream effectors remained unchanged. This demonstrates that specific activation by a Rho-GEF can dictate the signalling specificity of small GTPases. Similar transitions in signalling specificity may have occurred during fungal evolution. Cdc42 and Rac1 have changed their roles in various fungal species. This transfer of biological functions between Rho-GTPases may have also allowed complete loss of Rac1, which has occurred at least twice in the fungal clade.

270. The vacuolar membrane protein PRO22 from *Sordaria macrospora* is involved in septum formation in early sexual structures. Sandra Bloemendal[1], Kathryn M Lord[2], Kathrin Bartho[3], Ines Teichert[1], Dirk A Wolters[3], Nick D Read[2] & Ulrich Kück[1] [1]Department of General and Molecular Botany, Ruhr-University Bochum, Universitaetsstr. 150, D-44780 Bochum, Germany, sandra.bloemendal@rub.de [2]Institute of Cell Biology, University of Edinburgh, Rutherford Building, King's Buildings, Edinburgh EH9 3JH, UK [3]Department of Analytical Chemistry, University of Bochum, Germany.

The transition from the vegetative to the sexual cycle in filamentous fungi requires a multicellular differentiation process. For the homothallic ascomycete *Sordaria macrospora*, several developmental mutants are described. One of these mutants, pro22, produces only defective protoperithecia and carries a point mutation in a gene encoding a protein which is highly conserved throughout eukaryotes. Extensive microscopic investigations revealed that pro22 displays a defect in ascogonial septum formation, indicating that PRO22 functions during the initiation of sexual development. Live-cell imaging showed that PRO22 is localized in the tubular vacuolar network of the peripheral colony region close to growing hyphal tips, and in ascogonia, but is absent from the large spherical vacuoles in the vegetative hyphae of the subperipheral region. Our aim is to extend the functional analysis of PRO22 by identifying interaction partners *in vitro* via yeast two-hybrid and *in vivo* via tandem-affinity purification.

271. Fungal developmental networks: Control of fruiting body formation in *Sordaria macrospora.* Ines Teichert & Ulrich Kück Department for General and Molecular Botany, Ruhr-University Bochum, Universitaetsstrasse 150, 44780 Bochum, Germany, ines.teichert@rub.de

Fruiting body formation in filamentous ascomycetes is a complex differentiation process applicable as model for eukaryotic cell differentiation in general. Regulation of fruiting body formation involves a plethora of factors ranging from signaling components to transcription factors and metabolic enzymes, and is still not completely understood. In our studies, the homothallic ascomycete *Sordaria macrospora* serves as experimental system to gain a better understanding of the molecular mechanisms underlying fruiting body development. By complementation of sterile pro mutants, several proteins were identified that are essential for completion of the sexual life cycle. Since different PRO proteins localize to different compartments, protein-protein interaction studies were employed to link these proteins and to identify novel regulators of the sexual life cycle. Yeast-two hybrid and biochemical analyses hint to an extensive network regulating cellular differentiation in a fungal model system.

272. The Aspergillus nidulans UDP-galactofuranose transporter, UgtA: roles in wall structure, hyphal morphology, and conidiation. Sharmin Afroz (a), Amira El-Ganiny (a, b), Susan Kaminskyj (a) a) Dept Biology, Univ Saskatchewan, Canada; b) Microbiology Dept, Faculty of Pharmacy, Zagazig Univ, Egypt.

Galactofuranose (Galf) is the 5-member-ring form of galactose found in the walls of fungi including Aspergillus. UDP- galactofuranose mutase (ANID_3112.1) generates UDP-Galf from UDP-galactopyranose (6-member ring form). UgmA is cytoplasmic, so UDP Galf must be transported into a membrane bound compartment prior to incorporation into cell wall components. ANID_3113.1 (which we call UgtA) was identified based on its high amino acid sequence identity with GlfB, the UDP galactofuranose transporter in A. fumigatus. UgtA is not essential. The ugtAΔ strain, ASA1 has a phenotype similar to that of ugmAΔ: compact colonies with wide, highly branched hyphae. ASA1 conidium production and germination were reduced compared to wild type. SEM showed that some ASA1 metulae produced phialide triplets, rather than pairs. These phialides did produce nucleated conidia. TEM showed that ASA1 hyphal walls were more than three-fold thicker than wild type strains. Aspergillus nidulans ugtA is predicted to have five exons, which we confirmed by isolating and sequencing its cDNA. The UgtA predicted product is a 400 amino acid integral membrane protein likely to have 11 transmembrane helices. An A. nidulans strain with UgtA-GFP under the control of its constitutive promoter had a punctate GFP fluorescence pattern consistent with localization to the fungal Golgi equivalent. We are exploring possible interactions between UgmA and cytoplasmic loops of UgtA.

273. Quantifying the importance of galactofuranose in Aspergillus nidulans hyphal wall surface organization using atomic force microscopy. Biplab Paul (a), Amira El-Ganiny (b,c), Tanya Dahms (a), Susan Kaminskyj (b) a) Dept Chem&Biochem, Univ Regina, Canada; b) Dept Biology, Univ Saskatchewan, Canada; c) Dept Microbiol, Fac Pharmacy, Univ Zagazig, Egypt. bcpaul_bge@yahoo.com

Galactofuranose (Galf), the five-member ring form of galactose, is a minor component of Aspergillus walls. Strains deleted for Galf biosynthesis enzymes UgeA (UDP-glucose-4-epimerase) and UgmA (UDP-galactopyranose mutase) lacked immunolocalizable Galf, and had growth defects and abnormal wall structure. We used atomic force microscopy and force spectroscopy to image and quantify surface elasticity and adhesion of ugeAΔ and ugmAΔ strains and to compare them with two near-isogenic wild type strains, AAE1 and ugeBΔ. Our results suggest that UgeA and UgmA are important for cell wall surface subunit organization and wall elasticity. The ugeAΔ and ugmAΔ strains had larger surface subunits and lower cell wall viscoelasticity than those of AAE1 or ugeBΔ hyphae. Double deletion strains [ugeAΔ, ugeBΔ] and [ugeAΔ, ugmAΔ] had more disorganized surfaces than single deletion strains. Wall surface structure correlated with wall viscoelasticity for both fixed and living hyphae, with wild type walls being the most viscoelastic and the double deletion strains being the least. The ugmAΔ and particularly the [ugeAΔ, ugmAΔ] strain were more adhesive to hydrophilic surfaces than wild type. We propose that Galf is necessary for proper packing of cell wall components, so its loss gives rise to surface disorder, greater hydrophilic character and reduced viscoelasticity.

274. IRENI permits major advances in FTIR imaging of fungal hyphae. Kaminskyj S (a), Nasse M (b, c), Rak M (c), Gough K (d), Hirschmugl C (b, c) a) Dept Biology, Univ Saskatchewan, Canada; b) Univ Wisconsin Milwaukee; c) Synchrotron Radiation Center, Madison WI; d) Dept Chemistry, Univ Manitoba, Canada

Fourier transform infrared (FTIR) spectroscopy is used for non-invasive characterization of organic compounds including complex mixtures such as cytoplasm. Studies on rapidly frozen and dried fungal hyphae, using brilliant synchrotron IR sources, showed that fungal tips have lower content than subapical regions in the same cells, and that hyphal composition changes in response to environmental perturbation. Recently (in conjunction with other methods) we used an FTIR microscope with improved sensitivity, a globar IR source, and a 64 x 64 focal plane array (FPA) detector to document hyphal mannitol distribution. These studies were limited to ~ 6μm pixel size. Now, a unique synchrotron IR source called IRENI with 12 IR beamlines illuminating a single FPA detector permits diffraction-limited resolution. With IRENI, we can 1) collect data at 0.5μm x 0.5μm pixel definition, 2) characterize hyphal cytoplasm and exudate, 3) analyze hyphae as they grow in a moist chamber. Here, we compare wild type and single gene deletion strains of Aspergillus nidulans: A4 (used for the genome sequencing project), AAE1 (an nkuA strain with wild type hyphal morphology), and nkuA strains further deleted for ugmA or ugeA, key members of the galactofuranose biosynthesis pathway that have abnormal hyphal morphology and wall architecture. With this technology and these strains we are beginning to unpack the biochemical complexity of fungal tip growth.

275. A Comparison of the Role of the *Candida albicans* Recombinases Rad51 AND Dlh1. Larriba, G., García-Prieto, F., Gómez-Raja, J., and Andaluz, E. Área de Microbiologia. F. Ciencias. Universidad de Extremadura, 06071 Badajoz

We are analyzing the role of *RAD51* and *DMC1* (*DLH1*) orthologs of the pathogenic yeast *Candida albicans* in spontaneous homologous recombination (HR) and DNA repair. In *S. cerevisaie*, Rad51 operates in mitosis and meiosis, whereas Dmc1 is meiosis-specific. Gene targeting (GT) was analyzed by disrupting a non-essential gene (*SHE9*). When compared to wt CAI-4, the frequency of correct GT (*SHE9/she9::hisG-URA3-hisG*) was decreased by 80% and 40% for *rad51* and *dlh1* null strains respectively. By comparison, *rad52* and *rad51 dlh1* null mutants were refractory to GT. We conclude that Dlh1 can partially substitute for Rad51 and that at least a recombinase is needed for GT in *C. albicans*. Characterization of 50 Uri- segregants (5-FOA- resistant) derived from a *SHE9/she9::hisG-URA3-hisG* heterozygote indicated that most segregants (*SHE9/she9::hisG*) arose through an SSA event in CAI-4 (76%), *rad51* (69%) and *dlh1* (74%) null mutants, suggesting that Rad51 and Dlh1 are dispensable for SSA. The rest of the Uri- segregants exhibited the wt pattern (*SHE9*). In CAI-4, the *SHE9* genotype was generated exclusively by HR (local gene conversion, gene conversion plus crossover, or BIR). In *rad51*, regeneration of the *SHE9* pattern was due to chromosome loss (CL)(55%), chromosome truncation (18%), and HR (27%). In *dlh1*, most events were HR, but CL was found in 10% of the isolates. Also, *rad51* was significantly more sensitive to MMS, bleomycin, and camptothecin than wt, whereas *dlh1* behaved like CAI-4. Still, *dlh1* exhibited a slight increased sensitivity to UV light, that again was significantly lower that that shown by *rad51* strains. These results suggest that, as compared to Rad51, Dlh1 plays a minor role in DNA repair, HR events, and genetic stability during the mitotic cycle of *C. albicans*.

276. Plasma membrane-compartmentalized activity of *Aspergillus fumigatus* RasA is required for polarized growth and virulence. Jarrod R. Fortwendel, Praveen R. Juvvadi, Luise E. Rogg, and William J. Steinbach Duke University Medical Center, Durham, NC USA

Ras homologs are multifunctional proteins that are localized to specific sub-cellular membranes via post-translational addition of farnesyl and palmitoyl lipid moieties. Farnesylation of Ras directs the nascent Ras protein to the endomembrane system, whereas plamitoylation drives localization to the plasma membrane. This "compartmentalization" of activity allows for specificity in signal transduction. We have previously shown that deletion of *A. fumigatus rasA* causes slowed growth, malformed hyphae, and reduced cell wall integrity. However, the membrane distribution and the role of sub-cellular compartmentalization of RasA activity in these important cellular processes are unknown. To examine the distribution of RasA, a GFP-RasA fusion was expressed in the delta-*rasA* mutant background. GFP-RasA localized primarily to the plasma membrane of actively growing hyphae and septa. Expression of GFP-RasA in the delta- *rasA* background resulted in recovery of the wild type phenotype, indicating the fusion was functional. Inhibition of protein palmitoylation using 2-bromopalmitate caused hyphal deformation and reduced growth, as well as mislocalization of the GFP-RasA protein to internal structures. To further explore the role of palmitoylation, mutations in two conserved cysteine residues, which function as palmitoylation sites, were introduced to completely block RasA palmitoylation. The palmitoylation-deficient RasA mutant (RasA-P) displayed a decreased growth rate and hyphal abnormalities similar to the delta-*rasA* strain, as well as complete mislocalization of GFP-RasA from the plasma membrane. The delta- *rasA* and RasA-P mutants displayed similarly altered glucan and chitin staining, while TEM analysis revealed similar cell wall structural differences in both strains. Virulence was decreased for both mutants in a mouse model of invasive aspergillosis. Taken together, our data reveal the importance of plasma membrane-localized RasA activity in polarized morphogenesis and virulence of *A. fumigatus*.

277. The polo like kinase PLKA in *Aspergillus nidulans* is not essential, but plays important roles in vegetative growth and negatively regulates sexual development. Klarita Mogilevsky, Amandeep Glory, and Catherine Bachewich. Department of Biology,Concordia University, 7141 Sherbrooke St. West, Montreal, QC. cbachewi@alcor.concordia.ca .

The Polo-like kinases (Plks) are conserved, multi-functional cell cycle regulators that play additional roles in metazoan development. We previously identified *plkA* in *Aspergillus nidulans*, the only Plk investigated in filamentous fungi to date, and partially characterized its function through overexpression. We now report the *plkA* null phenotype. Surprisingly, *plkA* was not essential, unlike other fungal Plks. A subset of Δ*plkA* cells contained defects in spindle and chromosome organization, supporting some conservation in cell cycle function. However, septa were present, suggesting that PLKA is not a central regulator of septation like other Plks. The Δ*plkA* colonies were compact with multi-branched hyphae, implying a novel role for PLKA in hyphal morphogenesis. These defects were suppressed by high temperature or low benomyl concentrations, suggesting that PLKA functions in part through influencing microtubule dynamics. However, Δ*plkA* colonies also demonstrated benomyl and temperature-insensitive decreases in conidiation and precocious formation of Hulle cells. This represents the first example of a link between a Plk and development in fungi, and suggests that PLKA negatively regulates sexual reproduction through distinct mechanisms. Phylogenetic analyses suggest that PLKA and filamentous fungal Plks are related to the divergent metazoan PLK4, whereas yeast Plks group with metazoan PLK1-3. Thus, PLKA has some conserved functions, but may play additional novel roles in influencing morphogenesis and negatively regulating sexual development.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

278. Dynamics of actin and actin binding proteins during septum formation in *Neurospora crassa.* Mouriño-Pérez, Rosa R., Olga A. Callejas-Negrete, Diego L. Delgado-Alvarez, Ramón O. Echauri-Espinosa. Departamento de Microbiología, CICESE. Ensenada, Mexico. rmourino@cicese.mx .

Filamentous actin plays essential roles in filamentous fungi, as in all other eukaryotes, in a wide variety of cellular processes including cell growth, intracellular motility, and cytokinesis. We visualized F-actin organization and dynamics in living *N. crassa* via confocal microscopy of growing hyphae expressing GFP fusions with homologues of the actin- binding proteins fimbrin (FIM) and tropomyosin (TPM-1), a subunit of the Arp2/3 complex (ARP-3), coronin (cor1) and a recently developed live cell F-actin marker, Lifeact. All GFP fusion proteins studied were also transiently localized at septa: Lifeact-GFP first appeared as a broad ring during early stages of contractile ring formation and later coalesced into a sharper ring, TPM-1-GFP was observed in maturing septa, and FIM-GFP/ARP3/COR1-GFP labeled cortical patches formed a double ring flanking the septa. Our observations suggest that each of the N. crassa F-actin-binding proteins analyzed associates with a different subset of F-actin structures, presumably reflecting distinct roles in F-actin organization and dynamics during all the stages of septation. Actin is present since early stages of septum formation, the contractile force of the actomyosin ring is related to the presence of tropomyosin and it seems that there is a need of plasma membrane remodeling regards the presence of endocytic patches labeled by fimbrin, coronin and Arp2/3 complex.

279. G1/S transcription factor orthologues Swi4p and Swi6p are important but not essential for cell proliferation and influence hyphal development in the fungal pathogen *Candida albicans*. Bahira Hussein[1], Hao Huang[1], Amandeep Glory[1], Amin Osmani[1], Susan Kaminskyj[2], Andre Nantel[3,4] and Catherine Bachewich[1]. [1]Department of Biology, Concordia University, Montreal, QC. [2]Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan. [3]Biotechnology Research Institute, National Research Council of Canada, Montreal QC. [4]Department of Anatomy and Cell Biology, McGill University, Montreal, QC. cbachewi@alcor.concordia.ca .

The G1/S transition is a critical control point for cell proliferation, and involves essential transcription complexes termed SBF and MBF in *Saccharomyces cerevisiae*, or MBF in *Schizosaccharomyces pombe*. In the fungal pathogen *Candida albicans*, G1/S regulation is not clear. To gain more insight on its G1/S circuitry, we characterized Swi6p, Swi4p and Mbp1p, the closest orthologues of SBF (Swi6p, Swi4p) and MBF (Swi6p, Mbp1p) components in *S. cerevisiae*. The *mbp1*Δ/Δ cells showed minor growth defects, and similar sensitivity to hydroxyurea as control cells. In contrast, *swi4*Δ/Δ and *swi6*Δ/Δ yeast cells dramatically increased in size, suggesting a G1 phase delay, and *swi4*Δ/Δ cells were highly sensitive to hydroxyurea, implying a role in G1/S regulation. Consistent with this, Gene Set Enrichment Analysis (GSEA) of transcription profiles revealed that G1/S-associated genes were significantly enriched in cells lacking Swi4p and Swi6p, and suggested that Swi4p and Swi6p have activating and repressing activity. Intriguingly, *swi4*Δ/Δ *swi6*Δ/Δ and *swi4*Δ/Δ *mbp1*Δ/Δ strains were viable, in contrast to the situation in *S. cerevisiae*, and showed pleiotropic phenotypes that included hyphal cells. Consistently, GSEA identified strong enrichment of genes that are normally modulated during *C. albicans*-host cell interactions. Finally, Swi4p and Swi6p physically interact in a complex. Since SBF binding sites are lacking in the *C. albicans* genome, Swi4p and Swi6p may thus contribute to MBF activity. Overall, the data suggest that the putative G1/S regulatory machinery of *C. albicans* contains novel features, and underscore the existence of an important relationship between G1 phase and hyphal development in the pathogen.

280. Many mRNAs of *Aspergillus fumigatus* are asymmetrically localized in germlings. Ken Oda, Mara Couto-Rodriguez, Susan Cowden, John Kerry, Michelle Momany, Dept. of Plant Biology, Univ. of Georgia, Athens, GA 30602, USA (koda@plantbio.uga.edu)

A. fumigatus is the most common airborne pathogen causing fatal mycoses in immunocompromised patients. Polarized growth is one of the critical factors for establishing fungal pathogenesis, but little is known about the genes involved in early polar growth and their regulation. To understand the spatial distribution of polarity related mRNA, we performed spatial gene expression analysis of germlings. *A. fumigatus* Af293 was cultured in complete medium for 8hr which is the time just before septation. Tip, base, and conidium regions were captured by Laser Microdissection Pressure Catapulting (LMPC) and whole germlings were collected as a reference. Total RNA was extracted and a cDNA library was constructed for each region and for whole germlings. The quality of each cDNA library was confirmed by performing qRT-PCR for highly expressed genes. mRNA sequencing of each library was performed using a Next- Generation Sequencer (454 GS FLX). By comparing each region, we found that more than 1000 mRNAs are asymmetrically localized. To confirm mRNA localization, we performed Fluorescence in situ Hybridization (FISH) with some of the highly expressed genes in each region and found that their distribution was consistent with sequence results. These data suggest that many mRNAs are asymmetrically localized in tip, base, and conidium region of germlings.

281. Mutational Analysis of *Aspergillus fumigatus* Calcineurin A reveals critical domains required for its function *in vivo* and targeting to the hyphal septum. Praveen R Juvvadi, Jarrod R Fortwendel, Luise E Rogg, and William J Steinbach. Department of Pediatrics, Division of Pediatric Infectious Diseases Duke University Medical Center, Durham NC, USA.

Calcineurin, a conserved calmodulin-dependent protein phosphatase, is a heterodimer consisting of the catalytic (CnaA) and the regulatory (CnaB) subunits. It is known to play key roles in virulence, growth and stress responses of pathogenic fungi. Critically understanding the calcineurin pathway and identifying the residues indispensible for calcineurin activity *in vivo* will pave way for devising new drug targets for combating Aspergillosis. We previously reported that CnaA localizes at the hyphal septum implicating its importance for septum formation and conidiophore development. By constructing the delta-cnaA delta-cnaB double mutant strain of *A. fumigatus* and utilizing the dual fluorescent labeling technique we provide evidence on colocalization of CnaA-GFP and mcherry-CnaB fusion proteins at the hyphal septum. Surprisingly, while the CnaB-GFP fusion protein mislocalized to the cytosol in the absence of of cnaA, cnaA still localized to the hyphal septum in the absence of cnaB. By site-directed point mutagenesis of several residues in the catalytic domain, CnaB binding helix, and the calmodulin binding domain of CnaA, we identify critical domains essential for its function *in vivo* apart from the absolute requirement of complexing with CnaB for its function at the hyphal septum.

282. Evaluating the roles of the non-receptor GEF RIC8 using suppressor screens in *Neurospora crassa*. Patrick C. Schacht and Katherine A. Borkovich. University of California, Riverside.

RIC8 is a Guanine Nucleotide Exchange Factor (GEF) for Galpha proteins, but is neither a receptor nor membrane bound, making it an interesting exception to canonical G protein signaling. It is essential for asymmetrical cell division during embryogenesis as well as synaptic signaling in animals. *Neurospora crassa* is an ideal system for the study of *ric8*, as this gene is not present in *S. cerevisiae* or plants. Deletion of *ric8* induces severe pleiotropic effects and a nearly lethal growth phenotype in *N. crassa*. Using random mutagenesis, I have generated a suppressor mutant which partially recovers the wild-type phenotype in the *ric8* deletion background. Using SNP- CAPS I have identified a 210kb region containing the mutation. The mutant has been sequenced using next generation sequencing approaches and the data is currently being analyzed. In parallel, I am analyzing many genes predicted to be involved in RIC8 signaling for possible suppressor functions through creation of double knockout mutants. Data generated through analysis of these mutants will be used to confirm and extend our understanding of the pathways which regulated by RIC8. Funding provided by NSF and NSF IGERT Grant No. DGE 0504249.

283. A mutational analysis of the Neurospora crassa cell wall. Free, Stephen J., Abhiram Maddi, Ci Fu, and Asuma Tanaka. Department of Biological Sciences, SUNY University at Buffalo, Buffalo, NY 14260 email: free@buffalo.edu

A large number of cell wall proteins are encoded in the Neurospora crassa genome. Knockout mutants affected in 65 of these proteins have been carefully characterized. Knockout mutations in four of these genes have easily identified phenotypes. The Neurospora wsc-2 gene encodes a homolog of the yeast Wsc2p receptor protein that functions to activate the cell wall stress response pathway. The Neurospora wsc-2 mutant has a weakened cell wall and is affected in the asexual stages of the Neurospora life cycle. We identified three GPI-anchored cell wall proteins, ACW-13, CCG-6 and HAM-7, which are required for the formation of a normal cell wall. The ccg-6 mutant is has a weakened cell wall and is affected in the production of aerial hyphae and conidia. The acw-13 and ham-7 mutants have weak cell walls and are affected in both the asexual and the sexual stages of the life cycle. We have also demonstrated that alpha-1,3-glucan synthase mutants have an altered cell wall demonstrating the importance of alpha-1,3-glucan as a cell wall component.

284. Identification and characterization of genes required for cell to cell fusion in Neurospora crassa. Stephen J. Free, Ci Fu, Priyadarshini Iyer, Amrita Herkal, Julia Abdullah, and Angela Stout. Department of Biological Sciences, SUNY University at Buffalo, Buffalo, NY 14260 Email: free@buffalo,edu

A rapid high-throughput screening procedure was used to identify cell fusion (hyphal anastomosis) mutants in the *Neurospora crassa* gene knockout library. Mutants in twenty five cell fusion genes required for the formation of conidial anastomosis tubes (CATs) were identified and characterized. The identified cell fusion genes included fifteen genes that are likely to function in signal transduction pathways (*mik-1, mek-1 mak-1, nrc-1, mek-2, mak-2, cdc-42, pp- 2, so/ham-1, ham-2, ham-3, ham-5, ham-9, ham-10, mob-3*). The screening experiments also identified five transcription factors that are required for cell fusion (*adv-1, ada-3, ada-6, rco-1, snf-5*). Two genes encoding proteins likely to be involved in the process of vesicular trafficking were also identified as being needed for cell fusion during the screening (*amph-1, ham-11*). Three of the genes identified by the screening procedure, *ham-6, ham-7*, and *ham-8*, encode proteins that might function in mediating the plasma membrane fusion event. Two of the putative signal transduction genes, four of the transcription factors, the two putative vesicular trafficking proteins, and the three proteins that might function in mediating cell fusion were identified as cell fusion genes for the first time during the experiments. A model for how fusion between the two plasma membranes might occur is presented.

285. *Npc1* is involved in sterol trafficking in the filamentous fungus *Fusarium graminearum*. Andrew Breakspear[1], Matias Pasquali[2], Yanhong Dong[1], Karen Hilburn[3], and H. Corby Kistler[1, 3] [1]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108 USA; [2]Département Environnement et Agro-biotechnologies (EVA), Centre de Recherche Public-Gabriel Lippmann, L-4422 Belvaux, Luxembourg.; [3]USDA-ARS, Cereal Disease Laboratory, St. Paul, MN 55108 USA.

The ortholog of the human gene *NPC1* was identified in the plant pathogenic, filamentous fungus *Fusarium graminearum* by shared amino acid sequence, protein domain structure and cellular localization of the mature fungal protein. In human, the cholesterol-binding Npc1 protein localizes to the endosomal membrane, putatively functioning in lipid trafficking from the endosome. Mutations in human NPC1 lead to a fatal disorder, Niemann-Pick type C disease, associated with accumulation of endocytosed LDL-derived lipids in late endosomes. The *Fusarium Npc1* gene shares 34% amino acid sequence identity and 51% similarity to the human gene, has similar domain structure and is constitutively expressed, although up-regulated in ungerminated macroconidia and ascospores. GFP-tagged Npc1p localizes to the fungal vacuolar membrane. Cultures derived from a delta*npc1* mutant strain contain significantly more ergosterol than wild type cultures. Staining with the fluorescent, sterol binding dye filipin shows that ergosterol accumulates in vacuoles of the delta*npc1* mutant but not the wild type strain. The delta*npc1* mutant has a temperature dependent reduction in growth and sensitivity to the detergent SDS compared with the wild type strain, or the mutant complemented with wild type *Npc1*. The mutant also is significantly reduced in pathogenicity to wheat and significantly more sensitive *in vitro* to tebuconazole, an ergosterol biosynthesis inhibitor. Our results are consistent with the interpretation that Npc1p is important for normal transport of ergosterol from the vacuole, and is essential for proper membrane function under particular environmental conditions.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

286. Asymmetric RNA localization in filamentous fungi *Aspergillus fumigatus.* Mara Couto-Rodríguez, Susan Cowden, Ken Oda and Michelle Momany. Plant Biology Department, University of Georgia, Athens, Georgia 30602.

Filamentous fungi, such as *Aspergillus fumigatus*, are characterized by a highly polarized growth that occurs mainly by hyphal tip extension. Fungal tip growth involves many processes such as cell wall synthesis, vesicle transport, exocytosis and endocytosis. Even though there has been a reasonable amount of progress understanding these processes in the past few years, the exact mechanisms that regulate establishment and maintenance of polarity are not completely understood. Recent studies of highly polar cells from *Drosophila melanoganster, Candida albicans, Ustilago maydis* and others have demonstrated that RNA localization is used to restrict translation spatially and temporally. Consequently, we investigated asymmetric RNA localization in *A. fumigatus* . Laser microcapture combined with 454 sequencing done in our lab identified many of transcripts that appeared to be asymmetrically localized in polar A. fumigatus cells. In order to validate the level of asymmetry detected by 454 sequencing we performed Fluorescent in situ hybridization (FISH) . Transcripts from tip, base and conidia that showed the greatest asymmetry and the highest expression levels were chosen to synthesize digoxigenin labeled dsDNA probes for in vivo detection in *A. fumigatus* germlings. FISH experiments confirmed that many individual mRNA's are differentially localized to tip, base and conidium.

287. Pheromone-Receptor Signaling in Multiple *Candida* Species Indicates an Important Role for the Receptor C- terminal Tail in "Shmooing." Ching-Hsuan, Lin and Richard J. Bennett Department of Molecular Microbiology & Immunology Ching-Hsuan_Lin@brown.edu; Richard_Bennett@brown.edu

Candida albicans is recognized as having a novel mating cycle in which **a** and alpha cells must switch from the white state to the opaque state to become mating competent. Mating is dependent on pheromone signaling, and *C. albicans* **a** cells respond to alpha pheromone via Ste2, a G protein-coupled receptor. To define regions of the receptor important for transducing the pheromone signal, we constructed Ste2 mutants with cytoplasmic C-terminal tail truncations. Notably, mutants with completely truncated cytoplasmic tails were highly impaired in forming mating projections (shmoos), yet these mutants still show expression of the mating-specific genes, *FUS1* and *FIG1*. These experiments illustrate that the C-terminal domain is critical for "shmooing," but not for the activation of MAPK signaling and the transcriptional response to pheromone. We have also heterologously expressed Ste2 receptors from related species in *C. albicans*. Pheromone receptor genes from *C. tropicalis, C. parapsilosis, C. lusitaniae,* and *Lodderomyces elongisporus* were tested in a *C. albicans £Gste2* mutant. Ste2 receptors from *C. tropicalis, C. parapsilosis,* and *L. elongisporus* were partially active when expressed in *C. albicans*, while the Ste2 receptor of *C. lusitaniae* was inactive, even when challenged with its native alpha pheromone. Strikingly, however, expression of a chimeric receptor consisting of the *C. lusitaniae* Ste2 sequence fused to the *C. albicans* cytoplasmic tail evoked a response to *C. lusitaniae* pheromone and underwent significant shmooing. Overall, we show that the C-terminal tail of Ste2 is important for polarized growth in response to pheromone, and that engineered strains expressing chimeric receptors represent a powerful tool for identification of pheromones in *Candida* species.

288. Deletion mutants of *FgPKAC1* and *FgATF1* which encodes a catalytic subunit of cAMP-dependent protein kinase and a CREB family transcription factor in *Fusarium graminearum*. Hideaki Saisu[1], Kazuhiro Yamashita[1], Makoto Kimura[2], Akihiko Ichiishi[1], Makoto Fujimura[1]. [1]Faculty of Life Scienses, Toyo Univ, Gunma, Japan. [2]Env.Mol.Biol., RIKEN, Yokohama, Japan.

Fusarium graminearum causes head blight of cereals and trichothecene contamination in cereal grains. Production of fungal secondary metabolites including mycotoxin is influenced by environmental condition and morphological differentiation, implying the involvement of signal transduction pathways. We isolated two *FgPKAC1* and *FgATF1* disruptants in *F. graminearum* (MAFF 111233) by using homologous recombination: two genes encodes a catalytic subunit of cAMP-dependent protein kinase (PKA) and cAMP response element binding (CREB) family transcription factor, respectively. Although the knock-out mutants of catalytic subunit of PKA show the slow growth and the stimulated conidiation in many fungi, however, *FgPKAC1* disruptant and also *FgATF1* disruptant grew normally in *F. graminearum*. We previously reported that production of trichothecenes in rice medium were markedly reduced in the stress response MAP kinase *FgOs2* disruptant. Like *Neurospora crassa*, a fungicide fludioxonil stimulated the phosphorylation of FgOS2 and upregulated the gene expression of *FgCAT1, FgCCG1* and *FgGCY1* gene which encodes putative catalase, clock-controlled gene and glycerol dehydrogenase. ATF-1 is one of the transcription factor regulated by OS-2 MAP kinase in *N. crassa*. When *FgATF1* disruptant, and also *FgPKAC1* disruptant, were cultured in rice medium, trichothecene was detected by TLC as well as the wild type strain, suggesting that FgPKAC1 and FgATF1 is not essential for trichothecene synthesis.

289. Genetic analyses of centromere-specific histone H3 proteins from three ascomycetes in *Neurospora crassa*. Pallavi Phatale, Kristina M. Smith and Michael Freitag. Department of Botany and Plant Pathology, Department of Biochemistry and Biophysics, and Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331.

Centromere assembly and inheritance are dynamic and organism-specific. Protein complexes involved in kinetochore assembly contain signature proteins that are highly conserved in most eukaryotes, while other proteins, or certain domains, are divergent even between strains within one taxon. This predicts the existence of both conserved as well as divergent protein interactions during centromere and kinetochore assembly and maintenance. The “centromere identifier”, a centromere-specific histone H3 (CenH3) forms the platform for centromere assembly and is one of these bipartite proteins. It contains a hypervariable N-terminal region and a highly conserved histone fold domain (HFD). We previously showed that C-terminally tagged *Podospora anserina* CenH3 (PaCenH3-GFP) substitutes for Neurospora CenH3 (NcCenH3) in mitosis and meiosis. Replacement of NcCenH3 with *Fusarium graminearum* CenH3 (FgCenH3) supported only mitosis in Neurospora and tagging at the C-terminus resulted in defects in meiosis. Domain swapping experiments of the N-terminus of FgCenH3 with the HFD of PaCenH3 allows mitosis and meiosis, but chimeras with N-terminal NcCenH3 or PaCenH3 combined with the HFD domain of FgCenH3 were infertile or barren. Results from domain-swapping experiments suggest that only a few amino acids within the HFD are crucial during meiosis. There are only 16 differences between PaCenH3 and NcCenH3 in the HFD region. We propose that these differences play an important role during the assembly and inheritance of regional centromeres.

290. Cytoskeleton and polarized growth. R. Fischer, N. Takeshita, C. Seidel, N. Zekert Karlsruhe Institute of Technology (KIT), Dept. of Microbiology, Hertzstr. 16, D-76228 Karlsruhe, reinhard.fischer@kit.edu

The interplay between the actin and the microtubule (MT) cytoskeleton in polarized growth of fungi has recently been revealed. In *S. pombe*, Tea1 – a so-called cell end marker protein - is transported to the plus ends of MTs by the kinesin Tea2, and is delivered to cell ends by hitchhiking with growing MTs. Mod5, which is posttranslationally modified by prenylation, anchors Tea1 at the cell ends, where Tea1 recruits formin for actin assembly. We showed recently that the functions are essentially conserved in *A. nidulans*. However, we found that the cell end marker complex is not only required for the polarization of the actin cytoskeleton but also for temporary attachment of the MTs to the complex. We also discovered that the correct localization of the cell end marker complex depends on sterol rich membrane domains. Several genes, involved in the formation of these membrane domains are currently studied. Recently, there is increasing evidence that endocytosis plays an important role in polarized growth. We characterized two Unc-104 related motor proteins and discovered that one of them, UncA, which is involved in endocytic vesicle transportation, preferentially moves along a detyrosinated MTs. Deletion analyses revealed a stretch of 80 amino acids in the tail of UncA important for the recognition of the special MT. To understand the function of different MT populations in A. nidulans, the ratio between tyrosinated and detyrosinated alpha-tubulin in the cell is modified by different means.

291. What Role does Heterotrimeric G protein Signaling Play in Polarized Growth of *Neurospora crassa*? Carla Eaton and Katherine Borkovich Department of Plant Pathology and Microbiology, University of California Riverside, USA

Heterotrimeric G protein signalling plays a role in most major cellular processes in *Neurospora crassa*, including vegetative growth, asexual and sexual development. However, whilst there is considerable knowledge about the roles heterotrimeric G-proteins play in these processes, considerably less is known about the part these genes play in regulating cell morphology. To investigate what role these genes may play in cell morphology and polarized growth we have carried out microscopic analysis of the *N. crassa* G alpha (gna-1, gna-2 and gna-3), G beta (gnb-1) and G gamma (gng-1) mutants, and a non-receptor guanine nucleotide exchange factor mutant (ric8). RIC8 is a proposed positive regulator of GNA-1 and GNA-3 in *N. crassa*. Mutants were assayed for any changes in cell morphology, conidial germination and hyphal branching using DIC (differential interference contrast) and fluorescence microscopy. Preliminary results suggest that ric8, gna-1 and gna-3 mutants all exhibit defects in cell morphology suggestive of a loss of polarized growth. To further investigate the roles these genes play in *N. crassa*, fusions of GNA-1, GNA-2 and GNA-3 with TagRFP were generated, allowing us to visualize the subcellular localization of these proteins throughout the *N. crassa* lifecycle.

292. An AP-1-like transcription factor, NAP-1, regulates expression of *gst-1* and *mig-1* in *Neurospora crassa*. Masakazu Takahashi, Kazuhiro Yamashita, Masayuki Kamei, Akihiko Ichiishi, Makoto Fujimura. Faculty of Life Sciences, Toyo University, Gunma, Japan.

AP-1-like transcription factors play crucial roles in oxidative stress responses by regulating antioxidant genes in yeast and fungi. The deletion mutant of AP-1 like *nap-1* gene was only slightly sensitive to menadione and H_2O_2 in *N. crassa*, and NAP-1 was not required for the induction of the genes encoding superoxide dismutases and catalases. However, microarray and qPCR analysis revealed that at least 12 menadione- induced genes such as glutathione S transferase *gst-1* and 1, 4-Benzoquinone reductase *mig-1*, were regulated by NAP-1. In addition, NAP-1 present in cytosol was translocated into the nucleus 5 min after treatment with menadione, suggesting that NAP-1 is one of the major transcription factors in response to oxidative stress. Although we could not found the sequences similar to yeast Yap1-responsive element (YRE) in the promoter of the NAP-1 dependent genes, induction of the *gst-1* and *mig-1* gene was detected within 5 min and reached a maximum at 20 to 30 min after menadione treatment, suggesting of direct transcriptional control by NAP-1. The gel shift assay is performed by incubating a NAP-1 protein with the DNA fragments of the *gst-1* and *mig-1* promoter. NAP-1 purified using bacterial expression system bind to both promoters. Reporter assays suggest that the region between -500bp and -300bp upstream of ATG was essential for induction of *gst-1* and *mig-1* genes.

293. Maltose permease-encoding mRNA is cleaved in *Aspergillus oryzae*. Mizuki Tanaka, Takahiro Shintani, and Katsuya Gomi Graduate School of Agricultural Science, Tohoku Univercity, Japan

Eukaryotic mRNA is degraded by two degradation pathways: the 5' to 3' degaradation pathway by Xrn1 and the 3' to 5' degradation pathway by exosome-Ski complex. To investigate the mRNA degradation mechanism in filamentous fungi, we generated the disruptions of orthologous genes encoding mRNA degradation machinery in *Aspergillus oryzae*. Interestingly, the disruptants of *ski2* and *ski3*, components of Ski complex, showed the remarkable growth defect on minimal medium containing maltose or starch as a sole carbon source, whereas they normally grew on the medium with glucose or fructose as a sole carbon source. Northern blot analysis showed that the 3'-truncated fragment of mRNA encoding maltose permease (*malP*) was accumulated in Ski complex deficient mutants. Circularized RT-PCR analysis revealed that the *malP* mRNA was cleaved at a large stem-loop structure situated within the coding region. Since the 3'-truncated *malP* mRNA has no translational termination codon, it would be recognized by a certain ribosome releasing factor(s). We thus generated the gene disruptant of HbsA, ortholog of yeast Hbs1 identified as a recognition factor of aberrant mRNA in which ribosome was stalled in translation elongation. In a *hbsA* disruptant, the 3'-truncated *malP* mRNA was accumulated, and its degradation was suppressed. These results indicate that the *malP* mRNA is cleaved by endonuclease and the 3'-truncated *malP* mRNA is degraded rapidly by HbsA-dependent 3' to 5' degradation pathway.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

294. MUS-10, related to mitochondrial fusion and senescence, is associated with yeast Fzo1 homologue UVS-5 in *Neurospora crassa*. Kiminori Kurashima, Michael Chae, Shuuitsu Tanaka, and Shin Hatakeyama. Lab. of Genet., Fac. of Sci., Saitama Univ., Saitama, Japan.

The *mus-10* mutant of *Neurospora crassa* was originally isolated as a mutant which were sensitive to methyl methanesulfonate (MMS). Recently we showed *mus-10* gene encoded novel F-box protein and deletion of F-box domain caused phenotypes coincident with *mus-10* mutant, *i.e.* fragmented mitochondria, instability of mitochondrial DNA and senescent growth (Kato *et al*. 2010). Since MUS-10 protein was believed to be included in SCF E3 ubiquitin ligase complex, determination of target protein of this complex would bring beneficial information for maintenance of mitochondria. We focused FZO-1, Neurospora homologue of mitochondrial fusion regulator Fzo1, target protein of SCF complex in budding yeast. FZO-1 protein was co-purified with MUS-10 protein by immunoprecipitation experiment. We failed to make *fzo-1* gene disruptant, so *fzo-1* was thought to be essential gene. The *uvs-5* mutation has been mapped very closely to *fzo-1* and has reported its mutagen sensitivity and senescence phenotype. The shape of mitochondria in *uvs-5* mutant was quite resembled to those of mus-10 mutant. Furthermore, when introducing *fzo-1* gene into *uvs-5* mutant, it complemented dysfunction of mitochondria and mutagen sensitivity. The *uvs-5* mutant harbors amino acid replacement in GTPase domain in FZO-1 protein. Further relationship between *mus-10* and *uvs-5* is discussed.

295. Evidence for the existence of *nei/fpg* family DNA glycosylase/AP lyase dependent base excision repair in filamentous fungus *Neurospora crassa*. Hikaru Hashimoto, Shuuitsu Tanaka and Shin Hatakeyama. Laboratory of Genetics, Faculty of Science, Saitama Univ. Saitama, Japan.

Evidence for the existence of *nei*/*fpg* family DNA glycosylase/AP lyase-dependent base excision repair in filamentous fungus *Neurospora crassa* Base excision repair (BER) is an important cellular mechanism that removes damaged bases that could induce mutations by mispairing or lead to breaks in DNA during replication. Dysfunction of BER is cause for abnormality of the process of embryonic development and a cancer-predisposition in mammals. Toward uncovering the BER in living cell, we found that filamentous fungus *Neurospora crassa* was reasonable organism in following three biological aspects. First, the gene-knockout library, which contains 11,000 strains that almost of all genes in *Neurospora* are replaced by marker gene, is available. Second, unlike the other lower eukaryotes, *Neurospora* possess almost all BER genes, which are common in mammals and higher plants. Third, the most importantly, there is homologues of *nei*/*fpg* family DNA glycosylase/AP lyase in *Neurospora* that is often found in higher eukaryotes, bacteria, and a part of fungi. However, in contrast to the research field of bacteria, little is known about the role of *nei*/*fpg* homolog in BER in eukaryotic cells. We examined the function of *Neurospora crassa fpg* homolog by using conventional genetic approach and biochemical analysis. Indeed, we observed that *Neurospora fpg* functioned in repair of AP sites, which arose in the process of BER of methyl methanesulfonate-induced DNA damage. We also discuss about the genetic relationship of *fpg* homolog with other members of BER in *Neurospora*.

296. MGMT homolog and NER pathway are involved to repair of O^6-methylguanine adducts in *Neurospora crassa*. Yoshikazu Shimura, Shin Hatakeyama and Shuuitsu Tanaka. Laboratory of Genetics, Faculty of Science, Saitama Univ. Saitama, Japan.

O^6-methylguanine (O^6-meG) is a mutagenic and toxic lesion that is produced by some alkylating agents, including *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG). Many organisms possess O^6-meG-DNA-metyltransferase (MGMT) that detects O^6-meG and transfer the methyl group to itself. In *N. crassa* genome database, we found only one MGMT homolog. In MGMT homologs, there is a conserved active site containing a sequence of four amino acids, -Pro-Cys-His-Arg-, However, the sequence of the *N. crassa* homolog was -Pro-Trp-Gln-Arg-. When the *mgmt* KO mutant was treated with MNNG, it exhibited higher susceptibility and mutation frequency than the wild type strain. The purified *N. crassa* MGMT protein produced in *E.coli* bound to short oligonucleotides containing O^6-meG, but did not have transfer activity of the methyl groups. It is known that mutants, which defect in the nucleotide excision repair (NER) pathway, have apparent sensitivities to MNNG . We tested epistatic relationships between the *mgmt* mutation and the NER deletion mutations, *mus-38* and *mus-43*. While the NER single mutants were more sensitive to MNNG than the mgmt mutant, the double mutants showed nearly equal MNNG sensitivities as the *mgmt* mutant. These results indicate that the MGMT homolog binds to the O^6-meG lesions and its complexes are repaired by the NER pathway in *N. crassa* .

297. Expression and localization of G-protein coupled pheromone receptor Bar2 in the basidiomycete *Schizophyllum commune*. Erika Kothe, Elke-Martina Jung, Dominik Senftleben, Susann Erdmann

The homobasidiomycete *Schizophyllum commune* has a highly developed, tetrapolar mating system with more than 23,000 mating types occurring in nature. The mating system regulates sexual development by interaction of homeodomain transcription factors encoded in the A mating locus and a pheromone/receptor system encoded in the B mating locus. The B-regulated signaling of sexual development was subject of the present study resulting in new information on expression of B mating genes and localization of pheromone receptors. By means of real-time PCR, the expression level of the pheromone receptor gene bar2 and the pheromone gene bap2(2) was determined during a compatible mating interaction. The B mating genes are expressed at low levels in monokaryotic mycelium, in accordance with the mating type independent fusion of hyphae in S. commune. Increased expression levels during mating were found which ensure induction of B-regulated processes during mating. Localization of the Bar2 pheromone receptor in the cytoplasmic membrane was observed at higher levels in unfused clamp cells indicating a role in clamp fusion. The investigation of C-terminal receptor truncation could show a new phenotype of pheromone receptor mutations and indicated binding domains for intracellular, C-terminal receptor tail. The genome sequence was used to investigate signal transduction in different mating interactions. In addition, new receptor and pheromone genes were identified in the genomic sequence of S. commune which might by involved in pheromone response.

298. On the role of NOX-derived ROS during cell fusion in *Neurospora crassa*. Alexander Lichius and Nick Read, Edinburgh University, UK, alexlichius@gmail.com

Deletion of NADPH-oxidase-1 (NOX-1), its regulator NOR-1, and the associated GTPase RAC-1, resulted in a complete loss of cell fusion in *Neurospora crassa*, whereas deletion of NOX-2 did not, confirming functional separation between both isoforms. Although *nox-1* and *nor-1* cells retained the ability to form conidial anastomosis tubes (CATs) they were unable to chemotropically interact. Ectopic expression of fluorescent NOX-1 and NOR-1 fusion constructs rescued both mutant phenotypes, and their localisation to internal membranes and the cytoplasm, respectively, indicated a role for NOX-1-derived reactive oxygen species (ROS) in intracellular redox signalling. This notion was supported by the fact that CAT formation was selectively inhibited through the addition of micromolar concentrations of hydrogenperoxide, which left germ tube development unaffected. Visualization of superoxide accumulation in the tips of *nox-1* and *nor-1* germ tubes suggested that NOX-1 activity is dispensable for polarized growth, but has specific functions during CAT-mediated cell fusion. Deletion of the catabolic NAD-dependent glutamate dehydrogenase (GDH-1(NAD)) resulted in a phenotype very similar to *nox-1*, whereas absence of its anabolic counterpart NADPH-dependent GDH (GDH(NADPH)) produced no obvious phenotype. Taken together, this data suggests that activity of GDH-1(NAD) is required to replenish NADPH stores in order to fuel NOX-1-mediated ROS production which is essential to induce morphogenetic transitions leading to cell fusion.

299. Unraveling the biological activities of a bacterial metabolite using *Saccharomyces cerevisiae* and *Neurospora crassa* as model organisms. Danielle Troppens, Olive Gleeson, Lucy Holcombe, Fergal O'Gara, Nick Read[1] and John Morrissey. Microbiology Department, University College Cork, Ireland, [1]Institute of Cell and Molecular Biology, University of Edinburgh, UK

Secondary metabolites are a rich source of antimicrobial and other bioactive molecules, mainly due to the frequent capacity to affect metabolism and other cellular processes in non-producing organisms. We are interested in the secondary metabolite 2,4-diacetylphloroglucinol (DAPG), produced by some *Pseudomonas fluorescens* strains. It exhibits a broad spectrum of antimicrobial activity but little is known about its cellular targets or possible fungal resistance mechanisms. We are using two model organisms, *S. cerevisiae* and *N. crassa*, to address these questions. DAPG treatment impairs cell growth in both organisms and causes loss of mitochondrial membrane potential suggesting that electron transport is a target. A genome-wide screen revealed that alterations of several processes, such as protein biosynthesis and DNA repair, can confer resistance. We also found that in both *S. cerevisiae* and *N. crassa*, DAPG induces a transient cytoplasmic Ca2+ signal. Using an aequeorin reporter system to monitor the Ca2+ signal we show that it originates in the external medium but is not transported exclusively via known channels. In addition to providing information on the antifungal mode of action of DAPG, this work may have broader significance in understanding interactions between bacterial and eukaryotic cells.

300. The Num1 Protein of *Ustilago maydis* is Required for Polar and Filamentous Growth. Nikola Kellner, Kai Heimel and Joerg Kaemper Karlsruhe Institute of Technology (KIT), Institute for Applied Biosciences, Hertzstr. 16, 76187 Karlsruhe, Germany, nikola.kellner@kit.edu

In the corn smut fungus *Ustilago maydis*, sexual development is initiated by the fusion of two haploid sporidia, resulting in a filamentous growing dikaryon that is capable to infect the plant. Growth of the dikaryon requires an elaborate regulation of cell cycle, migration and distribution of the two nuclei and the polar growth of the hyphae. We have identified the Num1 protein with a pivotal function during these processes. Num1 is a homologue of SPF27, one of the four core components of the conserved Prp19/CDC5 splicing associated complex. Vegetative growth of sporidia is not altered in *num1* deletion mutants; however, the hyphae show various polarity defects, delocalized septae and dislocalized nuclei. Using the Yeast Two-Hybrid system, we identified CDC5, another conserved component of the Prp19/CDC5 complex, as Num1 interactor. Interestingly, we also identified proteins with functions during vesicle-mediated transport, in particular the kinesin 1 motor protein. The Num1/Kin1 interaction was verified by CoIP and Split-GFP analysis. Both *num1* and *kin1* deletion strains exhibit identical phenotypes with respect to vacuole morphology, filamentous and polar apical growth, corroborating the genetic interaction between Num1 and Kin1. Our data connect the splicing machinery and long distance transport in *U. maydis*. We will present our current view whether (and how) these two disparate mechanisms may be matched.

301. Heterokaryon Incompatibility Genes in *Aspergillus fumigatus*. Sean R Weaver[1], Nigel Dunn-Coleman[2], Maria R Diaz-Torres[2] and Geoff Robson[1+2] 1. The University of Manchester, United Kingdom. 2. AlerGenetica SL, Santa Cruz de Tenerife, Spain. www.alergenetica.com

Many filamentous fungi have developed a recognition system restricting hyphal fusion between members of the same species. Heterokaryon incompatibility (HI) is a self/non-self recognition system dependent on alleles at *het* loci. Incompatibility during hyphal fusion results in compartmentalisation of hyphal tips followed rapidly by localised programmed cell death (PCD). *Aspergillus fumigatus* is an opportunistic human pathogen able to cause fatal invasive infections and has been shown to undergo PCD under certain stresses including exposure to antifungal agents. An understanding of which genes play important HI roles in *A. fumigatus* could provide useful insight into potential apoptotic triggers and drug targets. To his end, compatibility groups are being defined through the use of pair-wise crosses of nitrate non-utilising *A. fumigatus* mutants generated from clinical and environmental isolates. Identified *het* domain genes have been knocked out in the genome-sequenced Af293 isolate using a PCR-fusion based technique to identify genes responsible for incompatibility. A transposon-based technique is also being used to interrupt random genes of *A. fumigatus* to generate a transposon library of spores that can be screened for compatibility grouping changes and which gene was responsible for any individual shift in behaviour. This work is funded by AlerGenetica SL and BBSRC.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

302. Imaging actin dynamics in *Aspergillus nidulans* using Lifeact. Brian D. Shaw, Laura Quintanilla, Srijana Upadhyay, and Zaida Hager Dept. Plant Pathology and Microbiology, Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX 77843-2132, USA

Polarization of actin to the hyphal apex is essential for hyphal growth. Previous work in *A. nidulans* has shown a sub-apical collar of actin::GFP patches that is associated with endocytosis and is necessary for growth. Here we use the Lifeact construct, an actin binding domain fused to either GFP or RFP, to image both actin patches and cables during growth. In addition to the sub-apical collar of patches in growing cells, we also note actin cables organized to the Spitzenkorper in growing tips. We also report here a new structure that we term the Sub-apical Actin Web (SAW). The SAW can be described as a complex array of actin cables distal to the tip in growing cells. This array is stable on the distal face but is highly dynamic on the proximal face with cables bending, retracting and growing toward the apex. Results of co-localization studies will be discussed.

303.Withdrawn

304. Characterization of a fungal-specific gene involved in cell death signaling in yeast. Myoung-Hwan Chi, Jun-Ya Shoji, Sumana Bhat, Jeremey Bell and Kelly D. Craven The Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA

Since it is established that baker's yeast, *Saccharomyces cerevisiae*, can undergo programmed cell death (PCD), it is considered a model organism to study fungal PCD because of its genetic simplicity and technical tractability. Several orthologs of mammalian PCD have been found in yeast and are involved in PCD, but no components specific for fungi have been identified thus far. Here we report a yeast gene, *Yapo1*, which might be involved in cell death signaling. Bioinformatic analysis revealed that the gene is unique in the fungal kingdom, and may have additional family members in filamentous ascomycetes. The *S. cereviseae* gene deletion mutant showed higher metabolic activity and survival rate than the wild-type or the complement strain in 1-2 mM hydrogen peroxide, which is known to induce PCD in yeast. The Yapo1 protein was localized to plasma membrane in normal conditions but translocated to intracellular organelles in oxidative conditions. Studies for uncovering the detailed mechanisms and PCD pathways related to *Yapo1* are in progress.

305. Chitosan is necessary to establish *Cryptococcus neoformans* infection. Lorina G. Baker Boomhower[1], Charles A. Specht[2], and Jennifer K. Lodge[1] Department of Molecular Microbiology[1], Washington University School of Medicine, 660 S. Euclid Avenue, Saint Louis, Missouri 63110, and Department of Medicine[2], University of Massachusetts, 364 Plantation Street, Worcester, Massachusetts 01605

Cryptococcus neoformans is an opportunistic pathogen that mostly infects immunocompromised individuals. Its cell wall is an essential organelle that provides structure and integrity. Several known virulence factors are located or attached to it, including melanin, phospholipase, and the polysaccharide capsule. The wall matrix is a complex structure composed of chitin, chitosan, and glucans. Chitin is an indispensable component with the majority converted to the deacetylated form, chitosan, by three chitin deacetylases (Cda1, Cda2, and Cda3). The deletion of all three-chitin deacetylease results in loss of chitosan production. In a mouse model the triple chitin deacetylse deletion strain was avirulent and did not establish infection. Additionally, both the chitin synthase three and chitin synthase regulator two deletion strains, each with negligible chitosan levels, had similar in vivo phenotypes. Together the data indicated chitosan is necessary for in vivo growth. Interestingly, the single deletion of *CDA1* resulted in attenuated virulence and reduced fungal burden, which suggested it or the chitosan produced by it is needed for virulence. Collectively the data suggest the proteins involved in chitosan synthesis may be good targets for anti-cryptococcal therapeutics.

306. Using mass spectrometry to identify proteins associated with a G alpha subunit in *Neurospora crassa.* Alexander Michkov and Katherine A. Borkovich Department of Plant Pathology and Microbiology University of California, Riverside

Many sensory and chemical stimuli are recognized by cell-surface receptors (GPCRs) that then use heterotrimeric G proteins to transduce this information to intracellular signaling pathways. Heterotrimeric G proteins consist of alpha, beta, and gamma subunits. Regulation is accomplished through the alternation between binding of GDP (inactive form) and GTP (active form) on the alpha subunit and dissociation of the alpha subunit and beta-gamma dimer. Both may regulate downstream effectors. *Neurospora crassa* has three G alpha subunits (GNA-1, GNA-2 and GNA-3), one G beta (GNB-1), and one G gamma (GNG-1). Mutation of *gna-1* results in reduced apical growth, osmotic sensitivity, premature conidiation and female sterility. We constructed a *Neurospora crassa* strain with tagged GNA-1, giving us the opportunity to isolate interacting proteins. Mass spectrometric analyses revealed ~50 putative interactors. Among the identified proteins are those involved in regulation of vacuolar and proteasomal protein degradation pathways and proteins with putative chaperone activity. This data suggests that GNA-1 interactome is highly complex and abundant, and includes not only effectors, but other elements of cell regulation.

307. Cellulolytic potential of thermophilic fungi -estimated with optimized assays for endo- and exoglucanases. Peter K. Busk, Mette Lange & Lene Lange Aalborg University, AAU Cph, Lautrupvang 15, 2750 Ballerup, Denmark E-mail: pkb@bio.aau.dk

The conversion of lignocellulose into glucose as basis for production of higher value products from crop residues and waste streams has both economic and environmental interest. Specific interest is associated with the urge for finding enzymes with high specific activity, good expression potentials and not the least, improved temperature stability. Based on this we chose to study the cellulytic potentials of thermophilic fungi. One critical factor for screening of microorganisms for cellulolytic potential is the availability of sensitive and simple cellulase assays. In the present study, we developed a modified endocellulase assay based on degradation of coloured carboxymethyl cellulose and a modified filter paper assay. The modified assays had up to ten fold higher sensitivity and were suitable for screening of small samples down to 1 µl. To find new cellulases we used the endocellulase assay on culture supernatants from thermophilic fungi grown on cellulose as sole carbon source. Several of the fungi showed high endocellulase activity and were further screened in the modified filter paper assay to test for overall cellulolytic potential. In conclusion, the two modified cellulase assays were suitable for screening of fungi for cellulolytic potential. The presentation will include further details of the discoveries made as well as an attempt to develop new discovery methods, unbiased by functional assays.

308. Analysis of Regulator of G protein signaling (RGS) proteins in *Neurospora crassa.* Ilva E. Cabrera and Katherine A. Borkovich. Department of Plant Pathology and Microbiology, Graduate Program in Cell, Molecular and Developmental Biology, and Video Bioinformatics IGERT, University of California, Riverside, CA 92521

Heterotrimeric ($\alpha\beta\gamma$) G proteins are essential components of signal transduction pathways that regulate environmental sensing, growth, and development in eukaryotes. Binding of a ligand to the G-protein coupled receptor (GPCR) causes an exchange of GDP for GTP on the Gα subunit. Consequently, this causes dissociation of Gα-GTP from the G$\beta\gamma$ heterodimer; both moeties have been shown to regulate downstream effectors. Regulators of G-protein signaling (RGS) proteins accelerate the hydrolysis of Gα-GTP, causing it to convert back to its inactive Gα-GDP state. *Neurospora crassa* contains five RGS proteins, RGS1-5. All five *rgs* knockout mutants' posses a hyphal growth defect. Microscopic analysis reveals that spore germination is affected in all *rgs* mutants and that *rgs- 1*, *rgs-2*, and *rgs-5* mutants have altered germ tubes. Formation of conidial anastomosis tubes is aberrant in certain mutants. Video bioinformatics approaches are being applied to further characterize cellular defects in all five *rgs* mutants in *Neurospora crassa*.

309. Hydrogen peroxide generates an increase in mitochondrial matrix free [Ca^{2+}]. Keith Fraser, Sairah Saeed, and Diana Bartelt. Dept. Biological Sci. St. John's Univ. 8000 Utopia Parkway, Queens, NY 11439.

Environmental stressors are known to lead to an increase in the concentration of [Ca^{2+}] in mitochondria. We have previously shown that exposure of the Aspergillus nidulans to hydrogen peroxide [H_2O_2] causes a dose-dependent increase in [Ca^{2+}]concentration in the mitochondria. Here we report that the increase in mitochondrial Ca2+ concentration in response to H_2O_2 is instantaneous, far exceeds the increase in [Ca^{2+}]in the cytoplasm and is unaffected by inhibitors of Ca2+ transport into the mitochondria. Isolated mitochondria also respond to H_2O_2 with a dose-dependent increase in [Ca^{2+}]concentration which is not inhibited by blockers of the calcium uniporter or mitochondrial transition pore which actually augment the increase in mitochondrial [Ca^{2+}]. Exposure of isolated mitochondria to H_2O_2 does not cause leakage of [Ca^{2+}]. The extent of mitochondrial swelling is H_2O_2 concentration dependent. These data are consistent with H_2O_2 causing a release of Ca^{2+} in the mitochondrial matrix itself that could initiate Ca^{2+}-dependent events leading to cell death. Supported in part by NIGMS (R15GM077345-01A1).

310. Dynein Heavy Chain Mutations Cause Multiple Mislocalization Phenotypes. Senthilkumar Sivagurunathan, Robert Schnittker, Stephen J. King, and Michael Plamann. School of Biological Sciences, University of Missouri-Kansas City, MO, USA. plamannm@umkc.edu

Cytoplasmic dynein is a multi-subunit, retrograde motor involved in a variety of cellular functions. The dynein heavy chain (DHC) subunit is responsible for ATP hydrolysis and interactions with microtubules and other subunits of dynein complex. The complex structural organization of DHC has made it difficult to understand its molecular mechanism. To gain insight into dynein function we utilized the ascomycete fungus Neurospora crassa as our model system. Mutations in components of dynein/dynactin pathway in Neurospora crassa result in curled hyphal growth morphology referred to as a ropy phenotype. Using a genetic screen we isolated spontaneous missense mutations in the DHC gene. The effect of DHC mutations on dynein localization was probed by replacing the native dynein intermediate chain (DIC) with a fluorescently tagged DIC. Wild type strains showed a bright hyphal tip localization of dynein with additional comet-like structures that exhibited behavior analogous to microtubule plus-end binding proteins. In contrast, DHC mutations exhibited one of several altered localization patterns of dynein. The array of cellular localization phenotypes suggests that dynein function can be perturbed in many different ways.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

311. Two Rac paralogs promote morphogenesis via the Ras1 signal transduction pathway in *Cryptococcus neoformans.* Elizabeth Ballou and J. Andrew Alspaugh. Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA.

The human fungal pathogen *Cryptococcus neoformans* grows in the lungs of immunocompromised individuals and has become an increasingly important cause of mortality in conjunction with the HIV/AIDS epidemic. This basidiomycete grows primarily as a haploid yeast in the host and the environment. Propagation in the host requires maintenance of the polar axis in the presence of temperature stresses and other host conditions. Previously we demonstrated that elements of the Ras1 pathway are required at 37˚C for the polarization of actin to the bud and for the maintenance of septal structures involved in cytokinesis. The polarity establishment protein Cdc42 is a potential downstream effector of Ras1 that has undergone an unusual duplication in the *C. neoformans* lineage. Both Cdc42 and its minor paralog Cdc420 are required for the recruitment and organization of structural proteins to the bud neck, activities important for thermotolerance and pathogenesis. In many filamentous fungi Cdc42 plays an overlapping role in morphogenesis with Rac proteins. We hypothesize that *C. neoformans* Rac proteins also play roles in hyphal and yeast morphogenesis. Similar to Cdc42, two Rac paralogs are present in the *C. neoformans* genome. Previously, we demonstrated that Rac1 is required for hyphal development during mating. Here, we use site directed mutagenesis to characterize the function of Rac2, examining its interaction with Cdc42, Cdc420, Rac1, and the Ras1 pathway. The *RAC2* gene is not essential, and preliminary evidence suggests that Rac2 plays a key role in both yeast and spore morphogenesis.

312. Global analysis of serine-threonine protein kinases in *Neurospora crassa.* Jacqueline A. Servin[1], Gyungsoon Park[1], Carol Jones[1], Gloria Turner[2], Lorena Altamirano[1], Patrick D. Collopy[3], Liande Li[1], Liubov Litvinkova[1], Hildur V. Colot[3], Carol Ringelberg[3], Jay C. Dunlap[3] and Katherine Borkovich[1] 1 Dept. of Plant Path. and Microbiol., University of California, Riverside, CA 2 Dept. of Chem. and Biochem., University of California, Los Angeles, CA 3 Dept. of Genetics, Dartmouth Medical School, Hanover, NH

Serine/threonine (S/T) phosphorylation is required for the transmission of crucial cellular signals. Ninety S/T protein kinase genes have been annotated in the *Neurospora* genome and knockout mutants have been generated for 79. While a significant number of S/T kinases have been studied, relatively little is known about the majority of these. To investigate the functions of S/T protein kinases, knockout mutants were subjected to a series of phenotyping assays. Fifty-seven percent of the strains exhibited defects in vegetative growth, asexual or sexual development. Among these, 71 percent displayed multiple defects and a total of 21 strains exhibited defects in all three processes, indicating that most S/T kinases regulate multiple functions. The knockout strains were subjected to a chemical screen utilizing a broad range of chemicals. Of the mutants analyzed, 32 percent displayed sensitivity or resistance to a chemical. Novel functions for six S/T kinases were inferred from the screen and two of these appear to suggest a link between calcium signaling and remodeling of the cytoskeleton.

313. Characterization of STE50, a MAPK adaptor protein that interacts with a novel GEF RIC8, in *Neurospora crassa.* James D. Kim[1,2], Ilva Cabrera[1,2], and Katherine A. Borkovich[1,2] Department of Plant Pathology and Microbiology[1], Graduate Program in Cell, Molecular and Developmental Biology[2], University of California, Riverside, CA 92521

RIC8 is a cytosolic guanine nucleotide exchange factor for G protein alpha subunits. We identified proteins that interact with RIC8 in *Neurospora crassa* by conducting a yeast 2 hybrid cDNA library screen. Among the interacting proteins is STE50. In *Saccharomyces cerevisiae* and *Magnaporthe grisea*, STE50 homologs have been shown to be an integral part of MAPK pathways. In order to investigate the role of STE50 in *Neurospora crassa*, we constructed a *ste50* knockout mutant and analyzed phenotypes. The results show that *ste50* mutants share some defects with the *ric8* and certain MAPK mutants. The results from MAPK phosphorylation assays implicate STE50 in regulation of multiple MAPK pathways. Taken together, these results suggest that STE50 regulates MAPK pathways in *Neurospora*.

314. Intercalary extension in vegetative hyphae facilitates colonisation of developing grass leaves by fungal endophytes. Christine R. Voisey[1], Suzanne J.H. Kuijt[1], Mike J. Christensen[1], Wayne R. Simpson[1], Kelly Dunstan[1], K.G. Sameera U. Ariyawansa[1], Nick D. Read[2], Neil A.R. Gow[3], Rosie E. Bradshaw[4], Hironori Koga[5]and Richard D. Johnson[1]. [1]AgResearch, Palmerston North, New Zealand.[2]University of Edinburgh, Edinburgh, Scotland.[3]University of Aberdeen, Aberdeen, Scotland.[4]Massey University, Palmerston North, New Zealand.[5]Ishikawa Prefectural University, Nonoichi, Ishikawa, Japan. christine.voisey@agresearch.co.nz

The highly polarised process of apical extension in vegetative hyphae is a distinguishing characteristic of fungal growth. However, an exception to this paradigm has recently been observed in endosymbiotic fungi that infect temperate grasses from seed. Grasses in the sub-family *Pooideae* form symbiotic associations with endophytic fungi of the genera *Epichloë* and *Neotyphodium*. The fungi colonise aerial tissues of developing grass seedlings by infecting the primordia of leaves and inflorescences as they develop on the shoot apical meristem. The hyphae of the endophyte are firmly attached to growing plant cells, and the two organisms are therefore committed to undertake coordinated developmental programmes.The leaves of grasses grow primarily though intercalary extension, a result of significant cell expansion throughout the leaf expansion zone. Conversely, vegetative fungal hyphae are thought to grow exclusively at the hyphal apex. In a striking example of co-evolution, these fungi have evolved a novel mechanism of elongation and division in intercalary compartments. This extremely rare mode of growth has enabled the attached endophyte to grow in synchrony with the host. The molecular and cytological events that orchestrate cell wall extension are the subject of a new study aimed at determining the specific orientation of the cytoskeleton and the movement of chitomes during polar and intercalary modes of growth. Two key fungal signalling pathways are currently being investigated to establish whether they participate in coordinating responses to mechanical stress which may trigger intercalary growth in endophytes when host cells expand.

315. The small GTPase SPGA plays a critical role in septation in the filamentous fungus *Aspergillus nidulans.* Hye-Ryun Kim, Tracy Zeng, and Bo Liu Department of Plant Biology, University of California Davis

Filamentous ascomycetes form mycelia of multinucleate hyphal cells. It is unclear how cytokinesis/septation is temporally regulated in these fungi. In Aspergillus nidulans, the kinase cascade of the septation initiation network (SIN) triggers the assembly and contraction of the actomyosin ring contraction at the septation site during cytokinesis. The spgA gene encodes a homolog of the small GTPase Spg1p which turns on the SIN pathway in fission yeast. Surprisingly, the null spgA mutation did not cause an obvious cytokinetic phenotype. In order to test whether SPGA acted as a trigger of cytokinesis, mutant forms of SPGA were expressed in the null spgA background. Over-expression of two constitutively active forms of SPGA, SPGAQ135L and SPGAD191A, did not cause an obvious phenotype in colony growth or conidiation when compared to wild type. But over-expression of the dominant negative form of SPGA, SPGAT108A, almost completely abolished conidiation. All three mutant forms of SPGA localized to spindle pole body as the wild type form. The two constitutively active SPGA forms induced cytokinesis to take place more frequently than wild type. When the dominant negative SPGAT108A was over-expressed, the SIN components were no longer detected at the spindle pole body and the septation site. Our results suggest that SPGA forms part of the trigger regulating the SIN pathway, and at least another small GTPase acts in parallel as SPGA.

316. Characterization of the Mutants *mtb-4A, mtb-4B* and Double Mutant in the Filamentous Fungus *Neurospora crassa*. Callejas-Negrete O. A[1], Plamann M. [3], Mouriño-Pérez R. R.[1], R.W. Roberson.[2], S. Bartnicki-García.[1]. [1]Department of Microbiology, CICESE, Ensenada, Mexico, [2]Department of Cellular and Molecular Biosciences, Arizona State University, Tempe, AZ, [3]School of Biological Sciences, University of Missouri-Kansas City, Kansas City, Missouri.

The *mtb-4A* and *mtb4-B* genes of *Neurospora crassa* have a high identity with the *nudF* gene of *Aspergillus nidulans*, the *pac1* gene of *Saccharomyces cerevisiae* and the human gene *lis1*. The proteins encoded by these genes appear to interact with the dynein/dynactin complex at the plus end of microtubules (Mts) and are required for proper nuclear distribution, in regulating Mts dynamics and cell growth. In *A. nidulans*, lack of NUDF may cause dynein to be kept at its inactive state and inhibit its transport of bound cargo, resulting in an over-accumulation at the plus end. The mammalian homolog of NUDF, LIS1, is product of a gene whose mutations cause brain malformation characterized by a disorganization of the neurons by a nuclear migration defect. We studied the effect of the lack of *mtb-4A* and *mtb-4B* during polarity establishment in *Neurospora crassa*. The lacks of *mtb-4A* and *mtb-4B* genes affect cell growth. The growth rate of *mtb-4A* mutant and double mutant *mtb-4A;mtb-4B* were 62% and 75% less than the wt, in contrast to *mtb-4B* mutant that had the same growth rate as the WT. Conidia production was affected in the mutants the strongest affect was in the double mutant (99% conidial production reduction). Branching rate in *mtb-4A* mutant and double mutant *mtb-4A;mtb-4B* were 3- folds higher than the WT (p mtb-4B mutant showed similar branching rate than the WT. The biomass production was the same in all mutants. The mutation in the *mtb-4A* and *mtb-4B* gene in *N. crassa* are not essential but in *mtb-4A* affects strongly the growth rate, increase the frequency of branching and reduce the production of conidia, although *mtb-4B* mutation does not affect the phenotype and growth rate, the lack of both *mtb-4A* and *mtb-4B* have a synergic effect.

317. Relationship between UV stress and expression of DNA repair genes in *Neurospora crassa*. Tsukasa Takahashi, Nami Hatakeyama, Akemi Kawakami, Makoto Fujimura, Akihiko Ichiishi. Faculty of Life Sciences, Toyo University, Gunma, Japan.

DNA is constantly damaged by endogenous and exogenous factors such as environments and chemicals. In these genotoxins, ultraviolet (UV) irradiation induces DNA damage such as cyclobutane pyrimidine dimers and 6-4 photoproducts. *N.crassa* has three mechanisms to repair UV-damaged DNA; nucleotide excision repair, UV dependent repair, photoreactivation. UV-induced DNA lesions are efficiently removed by these repair systems, thus *N.crassa* show highly resistance to UV. In human and *S.cerevisiae*, it has been reported that some of DNA repair genes involved in removal of UV-damaged DNA lesions were induced by UV irradiation. Furthermore, some MAP kinase pathways were activated in response to UV irradiation. In *N.crassa*, characterizations of DNA repair gene mutants have been performed in detail, but relationship between expression of these genes and UV stress are not clear yet. In this study, we investigated whether UV stress is involved in regulation of expression of DNA repair genes, and whether UV stress activates MAP kinase pathway like a human. We show that some DNA repair genes such as *mus-40, mus-43* were up-regulated by UV irradiation. The OS-2 MAP kinase was activated in response to UV irradiation, and then *mus-40, mus-43* were not induced after UV irradiation in *os-2* mutant. In addition, *os-2* mutant was more sensitive to UV irradiation than the wild-type. These date suggest that the induction of *mus-40* and *mus-43* gene by UV irradiation is regulated by OS MAP kinase.

318. New ways of looking at Neurospora recombination. Fred Bowring, Jane Yeadon , Kyle R. Pomraning#, Kristina M. Smith#, Michael Freitag# and David Catcheside. School of Biological Sciences, Flinders University, Adelaide, Australia; #Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA.

There are a few limitations of the methods routinely used to study Neurospora recombination. Firstly, while relatively easily obtained, chromatid data can be ambiguous. Secondly, although it is a simple matter to determine if a particular mutation alters the frequency of recombination, identifying any effect on the timing of recombination is problematic. Finally, it has not been practicable to study recombination at more than a few loci in a given cross. Here we report the results of our attempts to remove these limitations using a fluorescence-based recombination reporter system and massively-parallel sequencing. High quality recombination data from ordered octads can be obtained by simply scanning rosettes from a cross heterozygous for different GFP alleles. Furthermore, a cross between two mutant GFP alleles can be used to determine the timing of recombination. In this type of cross recombination can yield a wild-type GFP so it is possible to ascertain what stage in the developmental sequence nuclei first fluoresce. In order to do a genome-wide audit of recombination we are currently sequencing the genomes of several ordered octads. David.Catcheside@flinders.edu.au

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

319. Localization and Dynamics of the Polarisome Component bud-6 in *Neurospora crassa*. Ernestina Castro-Longoria and Mario E. Yañez-Gutierrez Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada. Ensenada B. C. México. E-mail: ecastro@cicese.mx

In fungal cells one of the protein complexes involved in polarity maintenance is the polarisome, which in *S. cerevisiae* consists of the proteins Spa2p, Pea2p, Bud6p, Aip3p, Bni1p and possibly Msb3p and Msb4p. In filamentous fungi there are few studies about the components of this complex. Therefore the aim of this study was to investigate the dynamics and localization of the protein BUD-6 in the filamentous fungus *Neurospora crassa* to elucidate its possible role in cellular morphogenesis. In mature hyphae the protein BUD-6 is located in the cellular apex as an apical gradient surrounding the Spitzenkörper (Spk) and its concentration decrease towards the sub-apex of hyphae. BUD-6 actively participates in the formation of septa in germlings and mature hyphae as well as in septa of conidiophores. Also it was possible to determine that BUD-6 plays an important role in cellular fusion or anastomosis. The *bud6* strain showed a drastic reduction in growth rate and was characterized by the lack of septa and cellular fusions, presented cytoplasmic leakage and produced abnormal conidiophores. Although hyphae of the *bud6* strain maintained a polarized growth, the typical hyphoid morphology was evidently altered and without the presence of a Spk. Therefore it can be concluded that in *N. crassa* the protein BUD-6 is not essential for polarity maintenance but plays a key role in the Spk integrity, septum formation, the anastomosis process, normal development of conidiophores and consequently normal colony growth.

320 Dissecting the role of the seven chitin synthases of *Neurospora crassa* in apical growth and septum formation. Rosa A. Fajardo-Somera[1], Robert W. Roberson[2], Salomón Bartnicki-García[1] and Meritxell Riquelme[1]. [1]Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada, CICESE. Ensenada, Baja California, México. [2]School of Life Sciences, Arizona State University, Tempe, Arizona, USA.

Fungal chitin synthases (CHS) are grouped into seven classes, four of them, III, V, VI and VII being exclusive of filamentous fungi. CHS classes V and VII have a myosin-like motor domain (MMD) at their amino terminus. Previous studies in *Neurospora crassa* showed that CHS-1, CHS-3, and CHS-6 tagged with GFP or mCherry accumulated at the core of the Spk, and also at nascent septa. We endogenously tagged with gfp the remaining chitin synthases genes, namely *chs-2* (NCU05239), *chs-4* (NCU09324), *chs-5* (NCU04352) and *chs-7* (NCU04350) to study their distribution in living hyphae of *N. crassa*. CHS-2, CHS-4, and CHS-7, appeared solely involved in septum formation. As the septum ring developed, CHS-2-GFP moved centripetally until it localized exclusively around the septal pore. CHS-5 was localized both at nascent septa and in the core of the Spk. We observed a partial colocalization of CHS-1-mCherry and CHS-5-GFP in the Spk. Total internal reflection fluorescence microscope (TIRFM) analysis revealed putative chitosomes containing CHS-5-GFP moving along wavy tracks. Collectively our results suggest that there are different populations of chitosomes, each containing a class of CHS. Mutants with single gene deletions of *chs-1*, *chs-3*, *chs-5*, *chs-6*, or *chs-7* grew slightly slower than the parental strain (FGSC#9718); only Δchs-6 displayed a marked reduction in growth. Both Δ*chs-5* and Δ*chs-7* strains produced less aerial hyphae and conidia. Currently, we are analyzing CHS activity and chitin content in all mutant strains to determine the relative importance of each CHS in cell wall biosynthesis.

321. Withdrawn

Biochemistry and Metabolism

322. Neurospora crassa is a Better Fungal Model System for the Study of Telomerase Regulation and Telomere Biology. Xiaodong Qi[1], Yang Li[1], Shinji Honda[2], Manja Marz[3], Steve Hoffmann[3], Peter Stadler[3], Eric Selker[2] and Julian J-L Chen[1] [1]Department of Chemistry and Biochemistry, Arizona State University, Tempe AZ 85287 [2]Institute of Molecular Biology, University of Oregon, Eugene, OR 97403 [3]Interdisciplinary Center for Bioinformatics, University of Leipzig, Germany

Telomerase is a ribonucleoprotein complex consisting of a catalytic telomerase reverse transcriptase (TERT) and a telomerase RNA (TER). It maintains nuclear genome stability by adding telomeric DNA repeats onto the ends of chromosomes using the integral TER as template. Due to the unusual diversity of size and sequence, TER has not been identified in filamentous fungi including *Neurospora crassa*, one of the most important model organisms. We hereby describe the identification and characterization of *Neurospora* TER, revealing structural elements and biochemical attributes conserved in *Neurospora* and vertebrate, but not in budding yeast. Using novel biochemical and bioinformatics approaches, we have successfully identified TER sequences from 55 filamentous fungi and cloned the gene from *N. crassa*. The secondary structure model of *N. crassa* TER, derived from a nucleotide-covariation analysis of the 55 sequences, showed structure features shared between vertebrate and fungal TERs, including a pseudoknot and a three-way junction. Unlike yeasts, *Neurospora* telomerase reconstituted *in vitro* was highly processive similar to vertebrate. Moreover, *Neurospora* telomerase synthesized the canonical telomere repeats (TTAGGG)n using a short 9 bases template of TER, a biochemical attribute conserved in ciliate and vertebrate telomerases. Conversely, telomerases from budding yeasts synthesized irregular DNA repeats using longer templates, i.e. 16 bases in *S. cerevisiae*. In addition, we have identified and cloned several telomerase and telomere associating proteins from *N. crassa*, and shown their interactions with telomerase and telomeres. Our results indicated that *Neurospora* telomerase shares more common features with vertebrates than budding yeast, and thus is a better model system for studying telomerase function and telomere biology.

323. Withdrawn

324. Insights into the specificity, transport mechanism and topogenesis of UreA, the specific urea transporter of *A. nidulans.* M. Sanguinetti*, S. Amillis§, C. Scazzocchio *˚, P.D. Dans# & A. Ramón* *Biochemistry Section, Dept. of Cellular and Molecular Biology, Fac. of Sciences, UdelaR Montevideo, Uruguay. §Dept. of Botany, Fac. of Biology, National and Kapodistrian Univ. of Athens and Dept. of Applied Genetics and Cell Biology, Univ. of Natural Resources and Life Sciences, Vienna-˚Dept. of Microbiology, Imperial College of London and Institute de Génétique et Microbiologie, Univ. Paris Sud, Orsay - #Biomolecular Simulations Group, Pasteur Institut of Montevideo. anacramonp@gmail.com

UreA is a high-affinity urea/H+ symporter which seems to be the sole active transport system specific for urea in *A. nidulans*. Orthologues have been identified and characterized in other fungi and plants. To address structure-function relationship in these transporters we attempted classical saturation mutagenesis and in parallel we designed a mutational strategy based on the identification of conserved residues in all known transporters of this group, together with 3D homology modeling of UreA. Conserved aminoacids localize in transmembrane segments predicted to be part of the binding and translocation domain. Site directed mutations introduced on an UreA::GFP fusion construct allowed us to identify a number of key residues involved in the recognition and/or translocation of urea across the plasma membrane. Other mutations, such as S446L, result in cellular mislocalization of the transporter. Second-site revertants of S446L, able to restore function, were isolated by chemical mutagenesis and are presently being characterized.

325. Galacturonic acid catabolism in *Botrytis cinerea.* Lisha Zhang and Jan A. L. van Kan Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands, E-mail: jan.vankan@wur.nl .

D-galacturonic acid (GalA) is the major component of pectin, which can be degraded by saprotrophic and pathogenic fungi; GalA potentially is an important carbon source for microorganisms living on decaying plant material. A GalA catabolic pathway was proposed in filamentous fungi, comprising three enzymatic steps, involving D-galacturonate reductase, L-galactonate dehydratase, and 2-keto-3-deoxy-L-galactonate aldolase. The *Botrytis cinerea* genome contains two non-homologous galacturonate reductase genes (*Bcgar1* and *Bcgar2*), a dehydratase gene (*Bcgdh1*), and an aldolase gene (*Bckdga1*). The four proteins were expressed in *E. coli* and enzymatic activity was confirmed. Targeted gene replacement of all four genes, either separately or in combinations, yielded mutants that were unable to grow on GalA as the sole carbon source. Mutants were also unable to grow on pectin or pectate, in spite of their ability to decompose the polymer by secreted pectinases. The mutants showed similar virulence as the wild-type strain on tomato leaves, apple fruit and bell pepper, whereas virulence was reduced on *Nicotiana benthamiana* and *Nicotiana tabacum* leaves. The results indicate that GalA serves as a very important carbon source for *B. cinerea* growth during infection on *Nicotiana* species, but not other plant tissues.

326. Exploring the link between NAD(P)(H) metabolism and pathogenicity in the rice blast fungus. Jessie Fernandez[1*] and Richard A. Wilson[1] [1]Department of Plant Pathology, University of Nebraska - Lincoln, USA *jfernandez99@huskers.unl.edu

To cause rice blast disease, *Magnaporthe oryzae* has to breach the surface of the host leaf and invade the plant tissue. Recent work has shown that trehalose-6-phosphate synthase (Tps1) monitors the nutritional status of the cell and regulates fungal virulence in *M. oryzae* via a novel NADP(H)-dependent genetic switch. Initiation of rice blast disease by this switch involves a G6P/ NADPH sensor protein (Tps1), NADP-dependent transcriptional co-repressor proteins and, uniquely, the non-consuming inter-conversion of NADPH and NADP acting as signal transducer. In addition, in response to G6P/ NADPH levels, the NADP(H)-dependent genetic switch controls the expression of a number of genes encoding NADPH-dependent enzymes. We sought to use our knowledge of the NADP(H)-dependent genetic switch to investigate how NADPH production and depletion is balanced in the cell, how crucial NADPH-requiring cellular processes are regulated by the availability of NADPH, and how these processes impact the ability of the fungus to cause disease. Through the functional characterization of genes involved in NADP(H) metabolism, we reported here an essential role for the non-oxidative pentose phosphate pathway enzyme transketolase in rice blast disease; we showed that the *de novo* NAD biosynthetic pathway is critical for cell viability and yet mutants of this pathway are remediated during plant infection; and we confirmed a link between the NADPH-dependent genetic switch and the expression of genes encoding enzymes of the glutathione and thioredoxin antioxidation systems – NADPH-dependent processes that are likely necessary for fungal colonization *in planta*.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

327. Biochemical and molecular characterization of *Trichoderma* strains isolated from different Brazilian agroecosystems: the antagonism potential against *Sclerotinia sclerotiorum*. Fabyano AC Lopes[1], Andrei S Steindorff[1], Alaerson M Geraldine[2], Valdirene N Monteiro[3], Murillo L Junior[2], Cirano J Ulhoa[1], Roberto N Silva[4] [1]Biological Science Institute, Goiania, Brazil [2]EMBRAPA-CNPAF, Santo Antonio, Brazil. [3]UnUCET-UEG, Anapolis, Brazil. [4]School of Medicine, University of Sao Paulo, Ribeirao Preto, Brazil. email: rsilva@fmrp.usp.br

The genus *Trichoderma* is a known agent of biological control of plant fungal pathogens. The control of these pathogens can occur by several mechanisms. The aim of this study was to evaluate the ability of antagonism against *Sclerotinia sclerotiorum*, Cell Wall Degrading Enzymes (CWDEs) production and metabolic profile of 21 *Trichoderma* strains isolated from different agroecosystems of Brazil. After molecular identification, *T. asperellum* showed more frequency followed by *H. lixii*, *T. tomentosum*, *T. koningiopsis*, *T. gamsii*, *T. erinaceum* and one strain could not be identified by ITS barcode. A cluster analysis of biolog results have separated all strains of *T. asperellum* from the other species although *H. lixii* showed two distinct groups and the unidentified strain was classified as *T. tomentosum*. The CWDEs did not show clustering between the species. No correlation between CWEDs production and antagonism was found, highlighting the complexity of the mechanisms of biological control by *Trichoderma*.

328. Oxido-reductive metabolism of L-arabinose and D-galactose in filamentous fungi: Metabolic crosstalk versus specific enzymes. Dominik Mojzita, Outi M. Koivistoinen, Kiira Vuoristo, Laura Ruohonen, Merja Penttilä and Peter Richard VTT Technical Research Centre of Finland, Espoo, Finland dominik.mojzita@vtt.fi

L-arabinose, the second most abundant pentose sugar, is used as a carbon source by a variety of microorganisms living on decaying plant material. Fungal microorganisms catabolize L-arabinose through an oxido-reductive pathway. We have identified two missing links in the pathway, L-arabinose and L-xylulose reductases in *A.niger*. D-galactose is a relatively rare hexose sugar in the plant cell wall mainly found in galactoglucomannan. There are three pathways indentified in fungi for D-galactose degradation; 1) the Leloir pathway in which D-galactose is phosphorylated, 2) the oxidative pathway which starts by an extracellular galactose oxidase reaction, and 3) a recently proposed oxido-reductive pathway which resembles the pathway for L-arabinose catabolism. It has been suggested in *T. reesei* and *A. nidulans* the oxido-reductive D-galactose pathway employs the enzymes from the L-arabinose pathway. It starts with the conversion of D-galactose to D-galactitol, probably carried by the xylose/arabinose reductase. The second step is catalyzed by L-arabitol dehydrogenase and the product of the reaction is an unusual sugar L-xylo-3-hexulose. We have identified the L-xylulose reductase possesses the activity with this intermediate which is converted to D-sorbitol. Finally, D-sorbitol is oxidized to D-fructose, which enters glycolysis. We have studied the pathway in *A. niger* and uncovered a more complex picture. Apart from showing the possible involvement of the L-arabinose pathway enzymes, we identified two dehydrogenases specifically induced on D-galactose, suggesting that *A. niger* might have specific genes for catabolism of D-galactose rather than using metabolic crosstalk suggested for *T. reesei* and *A. nidulans*.

329. Characterization of *Emericella nidulans* RodA and DewA hydrophobin mutants. Britt Guillaume Jensen*, Jakob Blæsbjerg Nielsen, Mona Højgaard Pedersen, Ib Søndergaard, Jens Christian Frisvad and Kristian Fog Nielsen. Department of Systems Biology, Technical University of Denmark, Denmark, *brgj@bio.dtu.dk

Hydrophobins are small amphiphilic proteins containing an eight cysteine pattern only found in filamentous fungi. They are involved in the attachment of hyphae to hydrophobic structures and the formation of aerial structures. Five *Emericella nidulans* mutant strains were examined to study the two hydrophobins RodA and DewA. Individual knock-out mutants *rodA* , *dewA* and the double deletion strain *rodA dewA* were constructed. Furthermore, two strains containing a point mutation in the first of the cysteines of RodA (*rodA-C57G*), where one was coupled to the *dewA* deletion, were included. The reference strain (NID1) and *dewA* displayed green conidia. However, *rodA* and *rodA dewA* showed a dark green/brown conidial pigmentation, while *rodA-C57G* and *rodA-C57G dewA* displayed lighter brown conidia. *rodA* and *rodA dewA* displayed a higher degree of hülle cells compared to the moderate amount observed for NID1 and *dewA* , while *rodA-C57G* and *rodA-C57G dewA* displayed a low number of hülle cells. NID1 and *dewA* conidia were dispersed as spore chains. *rodA* , *rodA dewA* , *rodA-C57G* and *rodA-C57G dewA* spores were associated in large clumps, where the conidia seemed to adhere to one another. The largest degree of spore clustering was observed for *rodA* and *rodA-C57G dewA* .

330. Identification of a novel secondary metabolite, regulated by LaeA in *Aspergillus flavus*. Saori Amaike[1], Ry Forseth[2], Anjali Choithani[1], Frank Schroeder [2], Nancy P Keller [1]. [1] University of Wisconsin-Madison, WI, USA [2] Cornell University, NY, USA

Aspergillus flavus is a saprophytic fungus able to act as a plant, animal, and human pathogen. The fungus' genome contains an array of secondary metabolic gene clusters, producing many secondary metabolites that may play a role in the fungus's biology. However, few secondary metabolites have been identified aside from aflatoxin, aflatrem and cyclopiazonic acid. The global secondary metabolite regulator, LaeA, regulates activation of many secondary metabolite gene clusters including aflatoxin, aflatrem and cyclopiazonic acid. Based on microarray data, LaeA regulates up to 22 secondary metabolite clusters in *A. flavus*, most of which have no known products. In pursuit of developing methodology to comprehensively assess the metabolic output of these secondary metabolite gene clusters, we deleted and over-expressed one non-ribosomal polypeptide synthetase (NRPS) gene, *nrpA* in *A. flavus*. Over- expressed *nrpA* produce three different novel compounds that are based on a disubstituted piperazine likely derived from tyrosine, consistent with involvement of *nrpA*. Here we present an overview of the possible biosyntheses of these compounds, the suspected encoding gene cluster and our present understanding of the role of this metabolite in *A. flavus* biology.

331. Role of flavohemoglobin homologous genes and nitric oxide in Aspergillus nidulans morphogenesis and mycotoxin production. Sachin Baidya, Jeffrey W. Cary and A. M. Calvo

Department of Biological Sciences, Northern Illinois University, Dekalb, IL

Flavohemoglobins are widely distributed proteins in both prokaryotic and eukaryotic organisms. These proteins contain a hemoglobin-like domain with a non covalently bound Heme B, and a reductase domain with binding sites for FAD and NAD(P)H. Its major physiological role is considered to be the reduction of nitric oxide (NO) concentration. Microorganisms appear to have evolved strategies for NO detoxification. Previous studies have shown that *Saccharomyces cerevisiae*, *Alcaligenes eutrophus*, *Escherichia coli*, and recently in *Aspergillus nidulans* flavohemoglobins have NO dioxygenase activity. Through a dioxygenase-mediated reaction, flavohemoglobin, in the presence of molecular O_2, converts NO into nontoxic nitrate ions. This confers resistance against nitrosative stress. In the present study we investigated the role of two flavohemoglobin homologous genes, *fhbA* and *fhbB*, in morphogenesis and in the production of the mycotoxin sterigmatocystin in *A. nidulans.* Deletion of *fhbA* resulted in lower levels of sterigmatocystin compared to the wild-type strain. On the other hand, supplementation of *A. nidulans* cultures with the NO-releasing compound, diethylenetriamine-NoNoate, resulted in an increase in toxin production and in the synthesis of other secondary metabolites, as well as an increase in cleistothecial formation.

332. Laccaria bicolor aquaporins: functions in soil growing hyphae and ectomycorrhizal symbiosis. Uwe Nehls[1,2], Sandra Dietz[2], Julia von Bülow[3], Eric Beitz[3], 1: University of Bremen, Faculty for Biology and Chemistry, Botany, Bremen, Germany 2: University of Tübingen, Microbiological Institute, Physiological Ecology of Plants, Tübingen, Germany 3: University of Kiel, Department of Pharmaceutical and Medicinal Chemistry, Pharmaceutical Institute, Kiel, Germany nehls@uni-bremen.de

Soil humidity and bulk water transport are essential for nutrient mobilization. Ectomycorrhizal fungi, bridging soil and fine roots of woody plants, are capable of modulating both by being integrated into water movement driven by plant transpiration and the nocturnal hydraulic lift. Aquaporins are integral membrane proteins that enable a concentration gradient driven flux of water and small uncharged ions over biological membranes. To gain insight into ectomycorrhizal fungal aquaporin function, we took advantage of the currently sequenced *Laccaria bicolor* genome. Here we present the first comprehensive study of a basidiomycotic aquaporin gene family, covering gene expression as well as protein function. Two aspects of aquaporin function were in focus of this investigation: water and solute permeability. While nearly all of the seven identified *L. bicolor* aquaporins mediated water permeability of *Xenopus laevis* oocyte plasma membranes, only three proteins revealed reasonable high rates for being of physiological significance. Protein function and gene expression data indicated these aquaporins to be mainly responsible for water permeability of fungal hyphae in soil and ectomycorrhizas. However, as growth temperature and ectomycorrhiza formation modified gene expression profiles of these aquaporins, specific roles in those aspects of fungal physiology are suggested. Moreover, two aquaporins, which were highly expressed in ectomycorrhizas, conferred plasma membrane ammonia permeability in yeast, pointing them towards being an integral part of ectomycorrhizal fungus-based plant nitrogen nutrition in symbiosis.

333. Effect of the *lea* gene on secondary metabolite production in *Aspergillus fumisynnematus.* Inhyung, LEE[1] and Sang Hee, KIM[2] [1]Dept. of Advanced Fermentation Fusion Science & Technology, [2]Dept. of Foods and Nutrition, Kookmin University, Seoul 136-702, Korea. leei@kookmin.ac.kr

The *laeA* gene is known to be a global positive regulator of secondary metabolite gene clusters in several filamentous fungi. To study the effect of the *lea* gene on secondary metabolite production in *Aspergillus fumisynnematus*, the *laeA* gene was cloned, disrupted and overexpressed. Sequencing revealed an open reading frame of 373 amino acids coding for a 41.6 kilodalton polypeptide with 95% sequence identity to LaeA of *Aspergillus fumigatus*. Like other LaeA, *A. fumisynematus* LaeA has a conserved protein methyltransferase SAM binding motif. The overexpression of the *laeA* gene under the *gpd* promoter changed the secondary metabolite production profile, suggesting that it activates some secondary metabolite gene clusters including silent ones in a normal culture condition.

334. Molecular analysis of secondary metabolite biosynthesis in *Alternaria alternata.* Ramona Fetzner1, Chris Lawrence2 and Reinhard Fischer3 1Karlsruhe Institute of Technology, Dept. of Microbiology, Karlsruhe, Germany Ramona.demir@kit.edu 2Virginia Bioinformatics Institute & Dept. of Biological Sciences,lawrence@vbi.vt.edu

Secondary metabolites are organic compounds that are not directly involved in normal growth, development or reproduction of organisms. In the mold *Alternaria alternata* a major group of secondary metabolites are mycotoxins. Important and in food and feed frequently found mycotoxins produced by *A. alternata* are the polyketides alternariol(AOH), alternariol-monomehtylether(AME) and altenuen(ALT). The polyketide-biosynthesis requires polyketide synthases, separated into groups dependent on the degree of reduction of their product. One example for a non-reduced polyketide is melanin. It is known that genes involved in the biosynthesis of polyketides are organized in gene clusters. This work describes the characterization of genes involved in secondary metabolites biosynthesis. In the *A. alternata* genome eleven putative polyketide synthases located within the corresponding gene clusters have been identified. One shows high similarity to melanin biosynthesis gene clusters of other organisms. Down regulation of the regulator within this cluster led to a whitish phenotype, and had a strong impact on polar growth. Additionally the formation of conidia is strongly reduced. This suggests that the regulator may not only control melanin synthesis but also other cellular processes. The analysis of four other polyketide synthases is under way.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

335. Analysis of the regulatory network involved in the production of a new secondary metabolite in *Fusarium fujikuroi.* Lena Studt[1,2], Bettina Tudzynski[2] and Hans-Ulrich Humpf[1] [1]Institute of Food Chemistry, WWU Münster, Corrensstr. 45, D-48149 Münster, Germany [2]IBBP, WWU Münster, Schlossgarten 3, D-48149 Münster, Germany l_stud01@uni-muenster.de

The filamentous fungus *Fusarium fujikuroi* is a notorious rice pathogen causing the "bakanae" disease due to the production of gibberellins (GAs), natural phytohormons resulting in hyperelongated and chlorotic primary leaves. Besides the production of GAs, *F. fujikuroi* produces a wide range of other secondary metabolites, such as the red polyketide bikaverin or the mycotoxins fusarin C and fumonisin. The genome of *F. fujikuroi* contains 17 polyketide synthases (pks) and until now only for a few of them their involvement in secondary metabolite production and the respective product is known. Recently, a new secondary metabolite in *F. fujikuroi*, a red polyketide, was identified in *F. fujikuroi*. We characterized the polyketide via mass-spectrometry and identified it as fusarubin (Fsr). It is a known secondary metabolite in other *Fusarium ssp.* (e.g. *F. graminearum*, *F. solani*). This is the first time Fsr was detected in *F. fujikuroi* and pinpointed to a concrete gene cluster in all *Fusarium ssp.* Currently, we are investigating the gene cluster and the regulatory network, e.g. the role of Velvet, pH, nitrogen and the G-Protein mediated signaling, that stands behind Fsr and its derivatives.

336. Efficient marker recycling: Application to construct double and triple mutants. Katarina Kopke, Alexandra Katschorowski, Birgit Hoff & Ulrich Kück Christian Doppler Laboratory for "Fungal Biotechnology", Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr- Universität Bochum, Universitätsstraße 150, 44780 Bochum, Germany, Katarina.Kopke@rub.de

To overcome the limited availability of marker genes in filamentous fungi we have established a marker recycling system. For this purpose the FLP/*FRT* recombination system from yeast was optimized to *Penicillium chrysogenum*. In a first step, we generated a nourseothricin resistance cassette flanked by *FRT* sequences in direct repeat orientation (*FRTnat1* cassette) and ectopically integrated this construct into a recipient strain. In a second step, a codon adapted recombinase gene was transferred into the strain, carrying the resistance cassette. The corresponding transformants showed to have lost the resistance marker by site specific recombination. The applicability of the developed system was further demonstrated in different ascomycetes. To further extend the application of the tool in *P. chrysogenum*, we constructed a DeltaPcku70FRT2 strain which enables the production of marker-free multiple deletion strains by efficient homologous recombination. For a one step approach we generated the *nat1*-Flipper, which contains the FLP/*FRT* system and the *nat1* marker gene in one construct. To regulate the recombinase gene expression the inducible *xyl* promoter was used. In future we intend to use different Flipper cassettes together with the DeltaPcku70FRT2 strain to construct marker-free double and triple mutants.

337. Comparison of homologous gene clusters for flocculosin and ustilagic acid synthesis in *P. flocculosa* and *U. maydis.* Beate Teichmann, Caroline Labbé, François Lefebvre and Richard R. Bélanger Département de phytologie, Centre de recherche en horticulture, Université Laval, Québec, G1V 0A6, Canada. E-Mail: beate.teichmann.1@ulaval.ca

Flocculosin and ustilagic acid are antifungal glycolipids produced respectively by the basidiomycetous fungi *Pseudozyma flocculosa* and *Ustilago maydis*. Both glycolipids consist of cellobiose, O-glycosidically linked to a long chain fatty acid. The molecules are further decorated with an acyl- and one or two acetyl groups. We have identified a gene cluster comprising 11 open reading frames responsible for flocculosin biosynthesis. In order to predict the biosynthetic pathway for flocculosin, we compared the cluster with the biosynthesis gene cluster for UA. As in *U. maydis*, the flocculosin gene cluster contains all genes necessary for the production of flocculosin including two cytochrome P450 monooxygenases (*cyp1* and *cyp2*), a complete single-chain fatty acid synthase (*fas2*), a glycosyl transferase (*fgt1*), an acyl- and an acetyl-transferase (*fat1* and *fat2*) as well as an export protein of the ABC-transporter family (*atr1*). The regulation of the gene cluster is mediated by a C2H2 zinc finger transcriptional factor (*rfl1*). Interestingly, the cluster contains an additional ORF (*fat3*) which shows homology to an acetyl-transferase and is missing the *ahd1* a gene involved in UA hydroxylation, two features that are likely responsible for the small structural differences between the two molecules.

338. Characterization of an *Aspergillus oryzae* cysteinyl dipeptidase expressed in *Escherichia coli*. Ryota Hattori[1], Mayumi Matsusita-Morita[1], Sawaki Tada[1], Junichiro Marui[1], Ikuyo Furukawa[1], Satoshi Suzuki[1], Hitoshi Amano[2], Hiroki Ishida[3], Youhei Yamagata[4], Michio Takeuchi[4], Ken-Ichi Kusumoto[1] [1]National Food Research Institute, Ibaraki, Japan, [2]Amano Enzyme Inc., Gifu, Japan, [3]Gekkeikan Sake Company Ltd., Kyoto, Japan, [4]Tokyo University of Agriculture and Technology, Tokyo, Japan.

Cysteinyl dipeptidase from *A. oryzae* (CdpA) was produced in *Escherichia coli* and purified. CdpA formed a homodimer and its molecular mass was determined as 109 kDa. CdpA-specific activity to Cys-Gly was 3.04 U/mg. The enzyme showed maximum hydrolyzing activity toward Ala-Cys, followed by Leu-Cys, Cys-Gly, Cys-Ala, and Gly-Cys among the cysteine-containing dipeptide substrates. Its substrate specificity was distinct from those of other cysteinyl dipeptidases of the M20 family. The activity of CdpA was increased by addition of Mn^{2+}, Zn^{2+}, and Co^{2+} at 0.1 mM. The activity was inhibited in the presence of Fe^{2+}. Several protease inhibitors reduced the activity. The complete inhibition of enzyme activity by EDTA indicates that CdpA is a metallopeptidase. It was optimally active at pH 7-8 and stable at pH 6-9 and at up to 40 • Ž. This study was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

339. Metabolome phenotyping of *Fusarium graminearum* wt and single gene deletion mutant strains affected in virulence under DON-inducing and non-inducing conditions. Rohan Lowe[1], Martin Urban [1], Gail Canning[1], William Allwood[1], Mike Beale[2], Jane Ward[2] and Kim Hammond-Kosack[1] [1]Centre for Sustainable Pest and Disease Management. [2] National Centre for Plant and Microbial Metabolomics; Rothamsted Research, Harpenden, Herts., AL5 2JQ, United Kingdom. Email: Martin.Urban@bbsrc.ac.uk

Fusarium graminearum causes plant disease on cereal crops including wheat, barley and maize. The fungus reduces yield and contaminates the crop with secondary metabolites toxic to plants and animals. These metabolites include trichothecene mycotoxins such as deoxynivalenol (DON). We recently reported on the use of metabolomic analysis to understand the basal metabolism in four Fusarium spp., *F. graminearum*, *F. culmorum*, *F. pseudograminearum* and *F. venenatum* under DON and non-DON inducing conditions (Lowe et al., MPMI, 2010, 16005-1618). In this study we investigated the global metabolic changes during time course experiments in the *F. graminearum* wt strain PH-1 for which the complete genomic sequence is available (http://www.broad.edu). Also, a 'triple-fingerprint' of analytical techniques including 1H-NMR and electrospray mass-spectroscopy (+/- ESI-MS) was recorded to characterise several single gene deletion mutants affected in mycotoxin biosynthesis, cell signalling and plant pathogenicity. Interestingly, all mutants analysed so far showed significant changes in primary metabolism. Understanding these changes will require more in-depth understanding of the metabolic networks and the characterisation of the metabolites present in *F. graminearum*. Rothamsted Research receives grant aided support from the Biotechnology and Biological Sciences Research Council.

340. Elimination of naphtho-[gamma]-pyrone production facilitates genome mining of secondary metabolites in *Aspergillus niger*. Yi-Ming Chiang[1,2], Kristen M. Meyer[3], Michael Praseuth[2], Scott E. Baker[3], Kenneth S. Bruno[3], Clay C. C. Wang[2,4] [1] Graduate Institute of Pharmaceutical Science, Chia Nan University of Pharmacy and Science, Tainan 71710, Taiwan, ROC. [2] Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, 1985 Zonal Avenue, Los Angeles, California 90089, USA. [3] Chemical and Biological Process Development Group, Energy and Environment Directorate, Pacific Northwest National Laboratory, Richland, Washington, 99352, USA. [4] Department of Chemistry, University of Southern California, College of Letters, Arts, and Sciences, Los Angeles, California 90089, USA. Email: K.B (bruno@pnl.gov) and C.W. (clayw@usc.edu)

Analysis of the *Aspergillus niger* genome sequence revealed the presence of over 50 core secondary metabolite genes capable of generating unique compounds. Identification and structural characterization of many of these predicted secondary metabolites are hampered by their low concentration relative to the major *A. niger* metabolites such as the naphtho-[gamma]-pyrone family of polyketides. The *alb1* PKS gene from *Aspergillus fumigatus* is known to be responsible for the biosynthesis of the naphtho-[gamma]-pyrone precursor of 1,8-dihydroxynaphthalene (DHN) melanin, a spore pigment. In the present study, we deleted a gene orthologous to *alb1* in *A. niger* strain ATCC 11414. Our results show that the *A. niger albA* gene is responsible for both the production of melanin pigment and of the profusion of naphtho-[gamma]-pyrones found in *A. niger* cultural extracts. The generation of an *A. niger* strain devoid of naphtho-[gamma]-pyrones will greatly facilitate the elucidation of cryptic biosynthetic pathways in this organism.

341. Biosynthesis of the Cyclic Peptide Toxins of *Amanita* Mushrooms. Hong Luo, Heather E. Hallen-Adams, John S. Scott-Craig, and Jonathan D. Walton. DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824.

Fungi in the basidiomycetous genus *Amanita* owe their high mammalian toxicity to the bicyclic octapeptide amatoxins such as α-amanitin. Amatoxins and the related phallotoxins (such as the heptapeptide phalloidin) are encoded by members of the "MSDIN" gene family and are synthesized on ribosomes as short (34- to 35-amino-acid) proproteins based on an effective combinatorial strategy. Confocal microscopy has revealed the cellular and subcellular localizations of amanitin accumulation in basidiocarps (mushrooms) of *Amanita bisporigera*. Consistent with previous studies, amanitin is present throughout the basidiocarp (stipe, pileus, lamellae, trama, and universal veil), but it is present in only a subset of cells within these tissues. Several lines of evidence implicate a specific prolyl oligopeptidase, *A. bisporigera* POPB (AbPOPB), in the initial processing of the amanitin and phallotoxin proproteins. The gene for AbPOPB is restricted taxonomically to the amatoxin-producing species of *Amanita* and is clustered in the genome with at least one expressed member of the MSDIN gene family. Immunologically, amanitin and AbPOPB show a high degree of colocalization, indicating that toxin biosynthesis and accumulation occur in the same cells and possibly in the same subcellular compartments. Through next generation sequencing, other promising candidate genes for posttranslational modification have been found in the vicinity of the known toxin genes, and their analysis is in progress.

342. Biosynthetic Loci of the Three Chemotaxonomic Markers in *Penicillium aethiopicum*: Viridicatumtoxin, Griseofulvin and Tryptoquialanine. Yit-Heng Chooi, Xue Gao, Ralph Cacho and Yi Tang Chemical and Biomolecular Engineering Department, University of California, Los Angeles, CA 90095. yhchooi@ucla.edu

Penicillium aethiopicum has three main chemotaxonomic markers: (**1**) viridicatumtoxin is a tetracycline-like compound with unique polyketide-isoprenoid hybrid origin; (**2**) griseofulvin is a classic polyketide antifungal drug with newly discovered antitumor potential; and (**3**) tryptoquialanine is an indole alkaloid with a complex multi-ring scaffold similar to the tremorgen tryptoquivaline. To search for the three corresponding biosynthetic gene clusters, a draft genome sequence of *P. aethiopicum* was obtained by 454 pyrosequencing technology. A total of 30 polyketide synthase (PKS) genes and 16 non-ribosomal peptide synthase (NRPS) genes were found in the genome, implying the undiscovered biosynthetic potential of this organism. Using a comparative and subtractive genomics approach, we were able to identify the biosynthetic loci for all of the three chemotaxonomic markers in *P. aethiopicum* genome. Targeted gene deletions showed that two unique non-reducing polyketide synthase (NR-PKS), which both lacks a C- terminal releasing thioesterase (TE) domain, are required for the biosynthesis of **1** and **2**. On the other hand, the biosynthesis of **3** involves an unusual tandem tri- and single modular NRPSs system. A combination of genetics and biochemistry approaches were employed to study the individual steps in the three biosynthetic pathways, which involved some remarkable biochemical transformations. Localization of the three gene clusters within conserved syntenic regions of the *P. aethiopicum* genome raises interesting questions regarding the evolution of clustering of secondary metabolite genes in fungi and provide insights to the underlying genetic basis of *Penicillium* chemotaxanomy.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

343. Chimeric production of flocculosin by *Ustilago maydis.* François Lefebvre, Beate Teichmann, Caroline Labbé, and Richard R. Bélanger Département de phytologie, Centre de recherche en horticulture, Université Laval, Québec, G1V 0A6, Canada. E-Mail : francois.lefebvre.3@ulaval.ca

Flocculosin is a glycolipid produced by the biocontrol agent *Pseudozyma flocculosa* and seemingly involved in the antagonism of *P. flocculosa* against members of the Erysiphales. Its structure is similar to ustilagic acid, a metabolite produced by *Ustilago maydis*. Based on the recent characterization of the ustilagic acid biosynthesis gene cluster, we were able to identify a homologous cluster in *P. flocculosa* sequenced genome. In this work, we hypothesized that the few differences found in the organization of the gene clusters explained the small structural differences between the two glycolipids. For instance, *P. flocculosa* possesses an extra acetyltransferase that would be responsible for the presence of an extra acetyl group in flocculosin. In addition, the carboxy terminal hydroxylations of flocculosin fatty acids in beta position would be caused by a single enzyme, Fhd1, while hydroxylations in beta and alpha positions in ustilagic acid would require two enzymes Uhd1 and Ahd1, respectively. To verify our hypothesis, we complemented wild-type and deletion strains of *U. maydis* and analyzed the expression profile of glycolipids of the transformants. Our results showed that Fat3 is responsible for the attachment an extra acetyl group on ustilagic acid molecules thus confirming its role.

344. The elucidation of the three-dimensional structure of ToxB, a chlorosis-inducing host-selective toxin produced by *Pyrenophora tritici-repentis*. Melania Figueroa[1], Afua Nyarko[2], Lynda M. Ciuffetti[1],Elisar Barbar[2]. [1]Department of Botany and Plant Pathology,[2]Department of Biochemistry and Biophysics. Oregon State University, Corvallis, OR 97331.

Pyrenophora tritici-repentis (Ptr), the causal agent of the disease tan spot of wheat, induces host cell death by secreting host-selective toxins (HSTs). Ptr ToxB (ToxB), a HST produced by this pathogen, induces chlorosis in sensitive wheat cultivars. ToxB is encoded by the gene *ToxB*, which translates into an 87 amino acid (aa) pre-protein. Cleavage of the signal peptide present in this pre-protein results in a 6.5 kDa mature ToxB protein. An allele of *ToxB*, *toxb*, encodes an inactive form of the toxin that is one aa longer than ToxB and differs in 13 aa from ToxB. In this study, we use nuclear magnetic resonance (NMR) spectroscopy to determine the structural basis for the activity difference between ToxB and toxb. Complete backbone and sidechain resonance assignments and secondary chemical shifts show that ToxB is predominantly a β-sheet folded protein, composed of six β-strands. Hydrogen isotope exchange experiments show that the protein is compact, rigid, and well-packed with slow exchanging core residues corresponding to the same residues of the β-strands. Interestingly, NMR spectra suggest that the structure of toxb differs considerably from ToxB. The structural comparisons between ToxB and toxb should aid in designing experiments that will define the mechanism of action, as well as functional structural components of ToxB.

345. Glu31, Asp142 and Asp171 of *Aspergillus oryzae* cutinase CutL1 are required for both interaction with hydrophobin RolA and consequent stimulation of polyester-degradation. Kimihide Muragaki[1], Kenji Uehara[1], Toru Takahashi[2,3], Youhei Yamagata[3] and Keietsu Abe[1,3*] [1]Grad. Sch. Agric. Sci., Tohoku Univ., Japan, [2]NRIB, Japan, [3]NICHe, Tohoku Univ., Japan [*]kabe@niche.tohoku.ac.jp

Hydrophobins are amphipathic proteins, and are ubiquitous among filamentous fungi. When the industrial fungus *Aspergillus oryzae* is cultivated in a submerged medium containing a biodegradable polyester polybutylene succinate-coadipate (PBSA), cutinase CutL1 and hydrophobin RolA are simultaneously secreted into the medium. RolA attached to the surface of PBSA particles specifically recruits CutL1, resulting in stimulation of PBSA hydrolysis[1)]. In our previous study, we studied amino acid residues involved in the RolA-CutL1 interaction by means of chemical modification and site-directed mutagenesis of RolA and CutL1. As a result, we found that His32 and Lys34 of RolA and Glu31, Asp142, Asp171 of CutL1 are involved in the RolA-CutL1 interaction. In the present study, to quantitatively elucidate the role of the three acidic amino acid residues of CutL1 in the RolA-CutL1 interaction, we characterized the interaction between CutL1 variants of the three residues and wild type RolA by using Quartz Crystal Microbalance (QCM). The QCM analysis revealed that replacement of the three acidic amino acid residues of CutL1 to serine caused increases in *K*D values for interaction with RolA. In conclusion, Glu31, Asp142 and Asp171 of CutL1 are critically required for the RolA-CutL1 interaction by multivalent effect.
1) Takahashi et al. Mol Microbiol. 57:1780 (2005)

346. Biosynthesis of 1403C from a marine Halorosellinia sp. (No. 1403), a novel anti-tumor anthraquinone via the polyketide pathway. Chuanpeng Niu, Menghao Cai, Xiangshan Zhou* and Yuanxing Zhang State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, PR China

1403C, a novel antitumor compound, was produced by a maine Halorosellinia sp. (No. 1403). In this paper the biosynthetic pathway of 1403C was unambiguously elucidated by feeding experiments with [1-13C]malonate and [1,2,3- 13C3]malonate followed by 13C nuclear magnetic resonance (NMR) spectroscopic investigation of isolated products. The results elucidated that 1403C was synthesized by polyketide pathway with 8 malonate both as starter units and extender units. After the elongation and cyclization, a O-methylation and decarboxylation as well as two oxidation and enoyl reduction reactions occurred respectively.

347. Withdrawn

348. Ammonium secretion during *Colletotrichum coccodes* infection modulates salicylic and jasmonic acid pathways of ripe and unripe tomato fruit. Noam Alkan[1,2], R. Fluhr[2], D. Prusky[1] dovprusk@agri.gov.il [1]Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, the Volcani Center, Bet Dagan, [2]Department of Plant Sciences, Weizmann Institute of Science, Rehovot, Israel

Insidious fungal infections by postharvest pathogens such as *Colletotrichum* remain quiescent after infection of unripe-green fruit. However, during ripening and senescence, the pathogens assume a necrotrophic life style, rapidly colonizing the tissue. *Colletotrichum coccodes* secretes ammonium during germination and colonization of host tissue. To further examine the role of ammonia in the infection process, transcriptome expression in fruit tissue treated with ammonia was compared to transcriptomes expression in infected tissue. The analysis revealed 82 and 237 common up-regulated and common down-regulated genes, respectively. Quantitative RT-PCR analysis of select transcripts in normal and transgenic NADPH oxidase antisense plants revealed that their expression was NADPH oxidase-dependent. Common-up-regulated genes showed over representation of salicylic acid (SA) dependent genes as well as genes related to biotic stress. The down regulated genes showed over representation of Jasmonic acid (JA) dependent genes. Indeed, direct application of SA to the fruit enhanced *C. coccodes* necrotrophic colonization, whereas the application of JA delayed colonization. Importantly, green fruit and red fruit displayed similar gene expression patterns although only red fruit is susceptible to colonization. It is thus likely that the resistance of green fruit to *C. coccodes* colonization is due to additional factors.

349. Production of terpenes with *Aspergillus nidulans.* Kiira Vuoristo, Kirsi Bromann, Mervi Toivari, Laura Ruohonen, Tiina Nakari-Setälä VTT Technical Research Centre of Finland Address: Tietotie 2, Espoo, P.O. Box 1000, FI-02044 VTT, Finland. Email: kiira.vuoristo@vtt.fi

Terpenes are a large and diverse group of hydrocarbons with many pharmaceutical and industrial applications including fragrances, preservatives, flavouring agents and drugs, *e.g.* anti-malarial artemisin, and the cancer drug Taxol. Terpenes with rearrangements in their carbon skeleton are referred to as terpenoids. The low water solubility, high volatility, cytotoxicity and complex structure of terpenoids make them difficult to produce in industrial scale. Modification of readily available precursors of terpenoids in filamentous fungi would present an alternative way to produce value-added compounds. There are not many reports on microbial transformation of terpenoids by fungi of the *Aspergillus* genus, although their ability to transform terpenes is recognised. The biotransformation of terpenes is of interest because it allows production of enantiomerically pure compounds under mild conditions. In nature, filamentous fungi produce terpenoids as secondary metabolites; *i.e.* they are not required for growth or development but instead function in communication or defence. The fungal terpenoids are involved in pathogenesis, production of toxins (e.g. alfatoxin), and utilization of specific carbon sources. Filamentous fungi have been widely used in industrial scale production of various compounds and especially *Aspergillus niger* and *Aspergillus nidulans* represent potential host organisms for the production and modification of various terpenoid products. This work describes genetic engineering of *A. nidulans* for terpenoid production. The aim is to study the potential of this organism for terpenoid production and isolate interesting terpenoid products or their precursors.

350. The *Cryptococcus gattii* proteome in growth and response to fluconazole. Hin Siong Chong[1], Leona Campbell[1], Ben Herbert[2], Elizabeth Harry[3], Mark Krockenberger[4], Marc Wilkins[5] and Dee Carter[1 1] School of Molecular Bioscience, Sydney University, Australia; [2] Chemistry and Biomolecular Sciences, Macquarie University, Sydney, Australia; [3] iThree Institute, UTS, Sydney, Australia; [4] Department of Veterinary Pathology, Sydney University, Australia; [5] School of Biotechnology and Biomolecular Sciences, UNWS, Sydney, Australia. dee.carter@sydney.edu.au

Cryptococcus gattii is a pathogenic yeast capable of causing disease in immunocompetent people. Antifungal susceptibility testing has found closely related strains of *C. gattii* can vary greatly in their susceptibility to fluconazole (FLC) and other azoles, without any apparent prior exposure to these drugs. Our group is interested in determining the molecular basis of the antifungal response in *C. gattii*. To establish a baseline for this work we have examined the proteome of *C. gattii* cells that are moderately susceptible to FLC during normal growth and in the presence of FLC. Compared to normal growth, cells treated with FLC had reduced levels of ribosomal proteins and increased stress-related proteins, including several heat shock proteins. A number of proteins involved in ATP biosynthesis also increased, indicating that ATP-dependent efflux of FLC and other toxic metabolic byproducts had been initiated. Ongoing studies will determine how the proteome compares in strains with elevated FLC resistance.

351. The complexity of polyketide synthase OrsA action in *Aspergillus nidulans.* Jakob B. Nielsen, Michael L. Nielsen, Christian Rank, Marie L. Klejnstrup, Paiman Khorsand-Jamal, Dorte M. K. Holm, Bjarne G. Hansen, Jens C. Frisvad, Thomas O. Larsen and Uffe H. Mortensen Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Søltofts Plads B221/B223, 2800 Kgs. Lyngby, Denmark

Polyketides (PKs) are chemically diverse molecules characterized by a broad spectrum of biological activities. Polyketide synthases (PKSs) are the main engines in the synthesis of PKs, and in fungi the PKS assembles small subunits to polymers in an iterative manner. Mapping of PKs to PKSs are not straight forward. The chemical architecture of the compounds produced depends on the primary structure and organization of the domains within the PKS molecule, and a majority of the PK products are also influenced by other gene products typically clustered in vicinity of the PKS. Moreover, activation of these clusters can be cumbersome, due to the clandestine regulation of the PK biosynthesis. One example is the gene cluster of PKS AN7909, *orsA*, in *Aspergillus nidulans*, which produces orsellinic acid and several other products. This synthesis has been provoked by several strategies. Here we report products that previously have not been linked to OrsA activity as well as cross-talk with the PKS AN7903 located approximately 20 kb downstream of *orsA*. These new observations emphasizes the challenges there are in uncovering the PK metabolome in fungi and the molecular genetics herein, which ultimately will facilitate rational engineering of the PK production apparatus.

352. A proteomic analysis on the effect of agmatine on *Fusarium graminearum* chemotypes. Pasquali M, Serchi T, Cocco E, Beyer M, Bohn T, Hoffmann L CRP Gabriel Lippmann, EVA Department, Belvaux, Luxembourg pasquali@lippmann.lu

Chemotype diversity of trichothecene production in *F. graminearum* was reported to potentially have consequences on the fitness of the isolates in the field. Agmatine was shown to be a potent toxin inducer of trichothecene B synthesis on an isolate belonging to the 15ADON chemotype. Our hypothesis is that toxin regulator signals may act differently among chemotypes. The effect of the compound on toxin production was measured on a set of 18 strains belonging to the 15ADON, 3ADON and NIV chemotypes of *F. graminearum in vitro* cultures using LC-MS/MS analysis. A linear mixed model analysis clearly identified a differential response of the chemotypes to agmatine stimulation. The nivalenol chemotype did not increase toxin synthesis significantly in response to agmatine while the other 2 chemotypes expressed a significant increase of DON and acetylated DON production ($P < 0.00001$). To understand the changes induced by agmatine among chemotypes at the proteomic level, three strains representative of the three chemotypes were then analysed using 2D-DiGE. Samples were obtained from agmatine and non-agmatine containing media after a growth period of 8 days at 22 ˚C and 150 rpm. Two-way ANOVA indicates that proteome changes were mainly determined by the effect of agmatine. Moreover, the interaction of strain and medium components played an important role for the protein profile. This approach allows identifying agmatine regulated proteins and agmatine chemotype-specific regulated proteins.

353. Isolation and cloning of trans-3- and trans-4-proline hydroxylase from the fungus Glarea lozoyensis. Loubna Youssar, Wolfgang Hüttel and Michael Müller. Institut of Pharmazie and Medicine Chemistry, Freiburg, Germany. E.mail: loubna.youssar@pharmazie.uni-freiburg.de

Glarea lozoyensis is an anamorph fungus, which was initially assigned Zalerion arboricola based on morphological traits. G. lozoyensis is of pharmaceutical interest, since it produces the antifungal secondary metabolite pneumocandin B0. This cyclic lipopeptide is chemically converted into a water-soluble derivative (caspofungin acetate) that is used against clinically relevant fungi pathogens. In Pneumocandin B0 trans-4- and trans-3-hydroxyprolines are incorporated which are derived from a hydroxylation of L- proline by proline trans-3-hydroxylase (P3H) and proline trans-4-hydroxylase (P4H), respectively. The P3H activity discovered in G. lozoyensis is unique and specific for pneumocandin B0 biosynthesis. We are interested in this new selectivity for biocatalytic applications, but also on the molecular and genetic level. To understand the physiological parameters that influence pneumocandin B0 and C0 production better, we proceed to clone the genes p3h and p4h by use of some conserved domains known from bacterial proline hydroxylases for designing degenerative primers. In future experiments, we want to express the genes heterologously in E. coli, knock them out in the native strain and study their expression, which will allow a deeper understanding of the mechanism of pneumocandin biosynthesis. Our approach includes genome sequencing and genomic library which are currently in progress and may will as a basis for screening.

354. Genome wide polyketide synthase gene deletion library in *Aspergillus nidulans.* Uffe H. Mortensen, Michael L. Nielsen, Jakob B. Nielsen, Christian Rank, Marie L. Klejnstrup, Dorte K. Holm, Katrine H. Brogaard, Bjarne G. Hansen, Jens C. Frisvad, Thomas O. Larsen Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Søltofts Plads B221/B223, 2800 Kgs. Lyngby, Denmark

Filamentous fungi produce a wealth of secondary metabolites that dramatically influence human life as they comprise both mycotoxins and pharmaceuticals. To forward the understanding of fungal secondary metabolism we have adopted a multidisciplinary strategy based on fungal ecology, analytical chemistry and molecular biology. Here we present an analysis of a library containing individual deletions of all known and putative PKS genes in the model fungus *Aspergillus nidulans.* The library has been challenged on a number of different media to uncover new links between genes and polyketide products. At our conditions, we detect several of the known products of *A. nidulans* including the PKS gene responsible for production of austinol. The validity of this conclusion is ensured by further mutagenesis including site directed mutagenesis of the locus as well as ectopic expression of the gene to indentify the first intermediate in austinol production. Based on our findings we call the gene *ausA*. Moreover, our results demonstrate several examples of crosstalk between different pathways in polyketide synthesis. To this end, it is important to stress that by investigating a genome wide deletion library at different conditions the chance of mis-assigning genetic links to products due to such crosstalk is dramatically reduced. Together the results and conclusions presented constitute our first step towards a systems understanding of the secondary metabolism of *A. nidulans*.

355. Heterologous expression of fungal polyketide synthases. Dorte K. Holm, Bjarne G. Hansen, Thomas O. Larsen, Uffe H. Mortensen Technical University of Denmark, Department of Systems Biology, Center for Microbial Biotechnology, Denmark.

Polyketides are a group of secondary metabolites that comprise molecules of very distinct structures and biological activities. In fungi, polyketides are synthesised via a multi-domain enzyme called a type I iterative polyketide synthase (PKS). As many polyketides are of medical or industrial use, various approaches have been addressed to link genes to known polyketide products e.g. deletion of genes encoding PKSs, overexpressing transcription factors, or using wide domain activators. Although these attempts have substantially increased our insights into polyketide biosynthesis, much is still to be learned especially in fungi where the full genome sequence is known, but where no efficient genetic tools exist. Based on a gene expression platform developed in our laboratory for heterologous or ectopic expression of genes in *A. nidulans*, we present an efficient approach for investigating polyketide synthesis in filamentous fungi. As a test case, 37 putative and known PKS encoding genes from the industrially relevant fungus *Aspergillus niger* were individually expressed in *A. nidulans* using *A. nidulans* promoters and terminators to ensure efficient transcription. This approach proved robust, fast, and efficient, and it is highly suitable for automating the generation of expression constructs for expression in *A. nidulans*.

356. A combined genetic and multi medium approach reveals new secondary metabolites in *Aspergillus nidulans.* Marie Louise Klejnstrup*, Morten Thrane Nielsen, Jens Christian Frisvad, Uffe Mortensen, Thomas Ostenfeld Larsen. Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark, *marlk@bio.dtu.dk

Secondary metabolites are a diverse group of metabolites which serve as important natural sources of drugs for treating diseases. The availability of full genome sequences of several filamentous fungi has revealed a large genetic potential for production of secondary metabolites that are not observed under standard laboratory conditions. Genetic approaches have proven a fruitfull strategy towards the production and identification of these unknown metabolites. Examples include deletion of the *cclA*[1] and *laeA*[2] genes in *A. nidulans* which affects the expression of secondary metabolites including monodictyphenone and terrequinone A respectively. We have deleted the *cclA* gene in *A. nidulans* and grown the mutants on several complex media to provoke the production of secondary metabolites. This resulted in the production of several metabolites not previously reported from *A. nidulans*. [1] Bok, J.W. *et al*, Nat. Chem. Biol., 5, 462-464 (2009). [2] Bok J.W. *et al*, Chem. Biol., 13, 31-37 (2006).

357. Heterologous expression of *Trichoderma reesei* polyketide synthases and non-ribosomal synthases in *Aspergillus nidulans*. Jorgensen, Mikael S.[1], Hansen, Bjarne G.[1], Skovlund, Dominique A.[2], and Mortensen, Uffe. H.[1]. [1]Center for Microbial Biotechnology, Technical University of Denmark, DK-2800 Lyngby, Denmark. [2]Novozymes A/S, DK-2880 Bagsværd, Denmark.

Polyketides (PKs) and non-ribosomal peptides (NRPs) constitute two classes of diverse secondary metabolites with a wide range of activities which can be both beneficial and disadvantageous from a human perspective. On one side PKs and NRPs include many toxins and virulence factors, but they also act as one of the main natural sources of medicines for treating diseases. PKs and NRPs are synthesized by large multi-functional proteins referred to as polyketide synthases (PKSs) and non-ribosomal peptide synthases (NRPSs). PKS and NRPS genes have been identified in many organisms such as bacteria, fungi and plants which therefore constitute a substantial pool of interesting gene candidates for production of new drugs. The lack of genetic tools for many of the original producers and the difficulty of linking the complex PKSs and NRPSs to their final PK or NRP product have led to the development of a heterologous expression system in *A. nidulans*. Many genetic tools are at disposal for *A. nidulans* and its metabolome has been well studied which is of great advantage when investigating expression of heterologous metabolite genes. A range of *T. reesei* PKS and NRPS genes have been heterologous expressed in *A. nidulans*. The results from these expressions will be presented.

358. Biobased Antibiotics From Basidios : Identification and manipulation of the pleuromutilin gene cluster from *Clitopilus passeckerianus.* S. Kilaru, C. Collins, A. Hartley, K. de Mattos-Shipley, P. Hayes, Andy M. Bailey* and Gary D. Foster* School of Biological Sciences, University of Bristol, Bristol, BS8 1UG, UK andy.bailey@bristol.ac.uk or gary.foster@bristol.ac.uk

With bacteria becoming resistant to antibiotics, there is a growing need to find new sources of antibiotics. Our work has focussed on the organism *C. passeckerianus* which produces a natural antibiotic, pleuromutilin. Recently, a derivative of pleuromutilin, retapamulin (developed by GSK) was approved for use in humans. Clinical trials have demonstrated its efficacy against certain Gram-positive bacteria including MRSA. We have developed all the tools to manipulate this important organism, and will present results on transformation, gene manipulation and enhancement, as well as gene isolation and mapping. These tools have allowed us to isolate the pleuromutilin gene cluster. Using the molecular tools we have been able to identify all genes involved, their roles, and perhaps most importantly, the ability to manipulate to elevate levels of antibiotic production and deliberately alter products produced. These results demonstrate that we are able to manipulate and control the *Clitopilus* genome. This provides a molecular toolbox which makes it possible to identify and manipulate individual genes of this fungus, and leading to some major new drugs which are not compromised by antibiotic-resistant strains of bacteria. The results will open up major opportunities for other previously intractable systems and antibiotics in fungi.

359. Sorbitol dehydrogenase as a part of *Aspergillus niger* D-galactose catabolism. Outi M. Koivistoinen, Peter Richard, Merja Penttilä, Dominik Mojzita VTT Technical Research Centre of Finland, Espoo, Finland outi.koivistoinen@vtt.fi

D-galactose is a hexose sugar found in hemicellulose. There are probably three different pathways for D-galactose catabolism in fungi, the Leloir, an oxidative and an oxidoreductive. The Leloir pathway has phosphorylated intermediates and galactokinase as the first enzyme. The oxidative pathway has no phosphorylated intermediates and D-galactose is oxidised to D-galactonate in the first reaction. In subsequent reactions D-galactonate is converted to pyruvate and glycerol. In addition to these two pathways an oxidoreductive pathway for D-galactose catabolism, that partly employs the enzymes of the L-arabinose pathway, was suggested to exist. In this work we studied the oxidoreductive D-galactose pathway where D-galactose is first reduced to D-galactitol by a reductase that is also active with L-arabinose. It is then oxidised to L-xylo-3-hexulose by the L-arabitol dehydrogenase. It was suggested that L-xylo-3-hexulose is then reduced to D-sorbitol which is finally oxidised to D-fructose by a sorbitol dehydrogenase. We identified a gene coding for a sorbitol dehydrogenase that is strongly induced on sorbitol and we evaluated the role of the enzyme in D-galactose catabolism.

360. GliT a novel thiol oxidase – implications in self resistance and biosynthesis of gliotoxin. D.H. Scharf[1], N. Remme[2], T. Heinekamp[1], P. Hortschansky[1], A.A. Brakhage[1,3], C. Hertweck[2,3] [1]Leibniz Institute for Natural Product Research and Infection Biology, Dept. Molecular and Applied Microbiology [2]Leibniz Institute for Natural Product Research and Infection Biology, Dept. Biomolecular Chemistry [3]Friedrich Schiller University of Jena, Germany

Aspergillus fumigatus and other pathogenic fungi have developed various chemical strategies to distress, weaken or even kill their plant or animal hosts. In invasive aspergillosis, the leading cause for death in immunocompromised patients, the fungal secondary metabolite gliotoxin plays a critical role for virulence. Gliotoxin is the prototype of a small family of epipolythiodioxopiperazines (ETPs), which features unique transannular di- or polysulfide bridges. Extensive molecular studies have revealed that this rare structural motif is indispensable for bioactivity and is the key to the deleterious effects of gliotoxin. Here, we describe the function of GliT, an enzyme of the gliotoxin biosynthesis pathway. We could reveal the activity of GliT both in vivo by means of feeding experiments and in vitro by heterologous overproduction and further biochemical characterisation of GliT. We proved that GliT is essential for biosynthesis of gliotoxin and therefore may play a critical role in virulence of *A. fumigatus*. Furthermore, GliT confers self resistance of *A. fumigatus* against gliotoxin. These investigations led to the discovery of an entirely new mechanism how microorganisms could prevent self poisoning by their own toxins. References: Scharf D. H., et al. (2010) J. Am. Chem. Soc. Schrettl M., et al. (2010) PLoS Pathog.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

361. Withdrawn

362. The additional D-galacturonate reductase genes in *Aspergillus niger* and *Hypocrea jecorina.* Joosu Kuivanen, Satu Hilditch, Dominik Mojzita, Merja Penttilä and Peter Richard VTT Technical Research Centre of Finland, Espoo, Finland joosu.kuivanen@vtt.fi

Pectin, a cheap and abundant raw material has a huge potential in white biotechnology. D-galacturonate, the main component of pectin, is catabolised to pyruvate and glycerol through a reductive pathway in eukaryotic microorganisms like filamentous fungi. This pathway also enables the utilization of pectin for the production of more valuable compounds. An example from that is the production of *meso*-galactarate (mucic acid) in metabolically engineered fungal strains. Enzymes functioning on the reductive D-galacturonate pathway are a D-galacturonate reductase, L-galactonate dehydratase, 2-keto-3-deoxy-galactonate aldolase and glyceraldehyde reductase. The genes coding for these enzymes in *Aspergillus niger* are *gaaA*, *gaaB*, *gaaC* and *gaaD*, respectively, while in *Hypocrea jecorina* (*Trichoderma reesei*) the corresponding genes are *gar1*, *lgd1*, *lga1* and *gld1*. *A. niger* also has a homologue gene sequence for *H. jecorina gar1* whereas *H. jecorina* has a homologue gene sequence for *A. niger gaaA* respectively. Functions of these two additional D-galacturonate reductase genes have remained unclear. We have now studied the roles of these two genes.

363. *Armillaria mellea* antibiotics – toward their mode of action and their role for interspecies communication. Markus Bohnert [1], Mathias Misiek [1], Volker Schroeckh [2], Hans-Martin Dahse [2], Fabian Horn [2], Dirk Hoffmeister [1 1] Friedrich-Schiller-Universitaet Jena, Department Pharmaceutical Biology at the Hans-Knoell-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany, markus.bohnert@hki-jena.de, dirk.hoffmeister@hki-jena.de [2] Leibniz-Institute for Natural Product Research and Infection Biology - Hans-Knoell-Institute, Beutenbergstrasse 11a, 07745 Jena, Germany.

The honey mushroom *Armillaria mellea* is a notorious pathogen and a producer of sesquiterpene orsellinate natural products, referred to as melleolides. *A. mellea* produces a great diversity of structurally related compounds, with some of them being strongly antibiotically active. We established a preliminary structure-activity relationship which identified the hydroxylation pattern and the position of the terpene double bond as critical elements for antibiosis. To learn more about the structural requirements and mode of action of melleolides, we carried out biochemical and transcriptomic experiments. Using *Aspergillus nidulans* as our target, transcriptomic data indicates that exposure to active melleolides impacts both basic cellular processes and secondary metabolism.

364. Functional analysis of the cryptochrome gene *cryA* of *Fusarium fujikuroi.* Marta Castrillo[1], Jorge García-Martínez[1], Richard Pokorny[2], Alfred Batschauer[2], Javier Avalos[1] [1]Departamento de Genética. Facultad de Biología. Universidad de Sevilla. Spain [2]Molekulare Pflanzenphysiologie. Philipps-Universität Marburg. Germany

In *Neurospora* and *Fusarium* neurosporaxanthin biosynthesis is induced by light. This photoresponse is achieved in *N. crassa* by the WC-complex, but unexpectedly not in *F. fujikuroi* and *F. oxysporum*, as shown by the photoinduction exhibited by the mutants of their *wc-1* orthologues, *wcoA* and *wc1*. Former data on the action spectrum for this photoresponse in other *Fusarium* species point to the mediation of a flavin-like photoreceptor. In addition to WC-1, the *Fusarium* genomes contain genes for a Vivid-like protein, a photolyase and a cry-DASH-type cryptochrome. Here we describe the functional analysis of this cryptochrome, that we named CryA. In contrast to *wcoA*, mRNA levels of *cryA* are induced by light. Targeted mutation of *cryA* produced light-dependent alterations in morphology and secondary metabolite production, as indicated by the abnormal accumulation bikaverin, a polyketide pigment. However, the null *cryA* mutants conserved the photoinduced accumulation of carotenoids, indicating the participation of a different photoreceptor. To learn more on its function, *cryA* was expressed in *E. coli* and the encoded protein purified close to homogeneity. *In vitro*, CryA is able to bind nucleic acids and repair cyclobutane pyrimidine dimers (CPD) in single-stranded DNA in a light-dependent manner. Its ability to bind DNA or RNA even without CPD lesions could provide the basis for a regulatory function of this cryptochrome.

365. Adenylyl cyclase plays a regulatory role in development, stress and nitrogen-controlled metabolism in *Fusarium fujikuroi.* Jorge García-Martínez[1], Attila L. Ádám[2], Javier Avalos[1] [1]Departamento de Genética. Universidad de Sevilla. Spain [2]Institute of Plant Protection. Agricultural Biotechnology Center, Szent István University, Hungary

Fusarium fujikuroi produces secondary metabolites of biotechnological interest, such as gibberellins, bikaverins, and carotenoids. Production of these metabolites is regulated by nitrogen availability and, in a specific manner, by other environmental signals, such as light in the case of the carotenoid pathway. A complex regulatory network controlling these processes is recently emerging from the different alterations of metabolite production found through the mutation of different regulatory genes. Here we show the effect of the targeted mutation of the *acyA* gene of *F. fujikuroi*, coding for adenylyl cyclase. Mutants lacking the catalytic domain of the AcyA protein show different phenotypic alterations, including reduced growth, enhanced production of a bikaverin-like compound, reduced production of gibberellins and a partially deregulated carotenoid biosynthesis. The phenotype is unexpectedly divergent in some aspects from that of similar mutants of the close relatives *F. proliferatum* and *F. verticillioides*: contrary to what was observed in these species, the *acyA*- mutants of *F. fujikuroi* show enhanced sensitivity to oxidative stress, and no change in heavy metal resistance or in the ability to colonize tomato tissue, indicating a high versatility in the regulatory roles played by cAMP in this fungal group.

366. Production of dicarboxylic acids by *Aspergillus carbonarius*, the engineering of a novel biochemical cell factory. Niels Bjørn Hansen, Mette Lübeck & Peter Stephensen Lübeck Section for Sustainable Biotechnology; Aalborg University Copenhagen. Lautrupvang 15, 2750 Ballerup, Denmark

The production of dicarboxylic acids by *A. carbonarius* is conducted under a WP involved in the European Commission's 7th framework supported Biorefinery project, SUPRABIO. SUPRABIO handles research, development and demonstration of sustainable production of fuels, chemicals and materials from biomass. For the economically and biological production of building blocks (BBs), the most promising BBs have been identified as four carbon 1,4 diacids; specifically the very high valued chiral acids. These acids have been shown to be fermented by several fungi, however, the yields and the productivity have not been shown to be substantial enough to sustain an industrial unit operation. Recent improvements in metabolic engineering have highlighted genomic modifications that increase the cytosolic flux of four carbon diacids in Yeast. Also, the sequencing of acid tolerant fungi unprotected by patenting restrictions opens up for novel cell factories. These issues and improvements lay the grounds for this WP that focuses on the utilization of C5- and C6 sugar biorefinery streams for the fermentation of high valued compounds. We have found a fungal strain that form the basis organism for the development of a novel cell factory by genomic changes that enhance production of defined compounds. Currently progress involves genomic manipulation, biorefinery side stream adaptation, and characterization of genetic changes in *A. carbonarius*.

367. Fumonisins in Aspergillus niger: Industrial and Food aspects. J. C. Frisvad *, K.F. Nielsen, J.M. Mogensen, U. Thrane, and T. O. Larsen Department of Systems Biology, Technical University of Denmark * jcf@bio.dtu.dk

Fumonisins are very toxic secondary metabolites from *Fusarium*, that was recently discovered in *Aspergillus niger*. *A. niger* (and its synonyms *A. awamori* and *A. lacticoffeatus*) is the only species in *Aspergillus* producing fumonisins, but only B2, B4 and B6. In contrast to *Fusarium verticiilioides* and other Fusaria producing fumonisins, *A. niger* is a generalist, and thus a lot of different foods can be contaminated with fumonisins. Fumonisins have already been detected in coffee, wine, grapes and raisins. However also in contrast to *Fusarium verticillioides*, *A. niger* produce fumonisins optimally on media with a high content of carbohydrates, and thus the many different media used in citric acid production, including apple, beet, citrus waste etc. are prone to fumonisin contamination. We have tested all available industrial strains of *Aspergillus niger* and they were all able to produce fumonisins and some of them also ochratoxin A. Some strains tested also produced fumonisins under citric acid production conditions. *A. niger* strains used for other purposes, including enzyme production, as transformation hosts etc. also produced fumonisins, at least in pure culture. Therefore *A. niger* is a serious problem for the industry, and different approaches to solve the problem will be mentioned.

368. Withdrawn

369. *Fusarium sulphureum* Δ*tri101* mutants overproduce the toxic metabolite FS-4. Gerlinde Wiesenberger[1], Mehrdad Shams[1,2], Rudolf Mitterbauer[1], Heidemarie Hellmer[1], Franz Berthiller[2], Christian Hametner[3], and Gerhard Adam[1] [1] Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria. [2] Center for Analytical Chemistry, IFA Tulln, BOKU, Tulln, Austria. [3] Institute for Applied Synthetic Chemistry, Vienna University of Technology, Vienna, Austria. gerlinde.wiesenberger@boku.ac.at

The *Fusarium sulphureum* strain MRC514 was used to generate *tri101*Δ knockout mutants. *TRI101* encodes an acetyltransferase implicated in the self protection mechanism of the toxin producer. The *tri101*Δ knockout strains of the diacetoxyscirpenol (DAS) producer MRC514 accumulated the expected trichothecene biosynthesis intermediates as determined by LC MS/MS and unknown substances. One major peak purified by preparative HPLC was identified by NMR spectroscopy to correspond to FS-4. The structure of FS-4 is related to trichothecenes but unusual because it lacks the epoxy group. Testing inhibition of eukaryotic protein biosynthesis using a wheat germ in vitro translation system revealed that FS-4 was surprisingly active. Using pdr5 toxin sensitive yeast we could show that a mutation in ribosomal protein L3 ($RPL3^{W255C}$) conferring resistance to trichothecenes also confers resistance to FS-4. Funded by FWF SFB F3702 and F3706

370. Impact of glucose metabolism during pathogenesis of *Aspergillus fumigatus.* Christian B. Fleck[1], Ilse D. Jacobsen[2], Silvia Slesiona[1,2] and Matthias Brock[1] [1] Microbial Biochemistry and Physiology; [2] Microbial Pathogenicity Mechanisms Leibniz Institute for Natural Product Research and Infection Biology, -Hans Knoell Institute-, Beutenbergstr. 11a, 07745 Jena, Germany Contact: Matthias.brock@hki-jena.de

Aspergillus fumigatus is the major cause of life-threatening invasive bronchopulmonary aspergillosis. Manifestation and progression of infection in the immunocompromised host requires the efficient uptake and metabolism of nutrients. However, nutrient sources available and used by the pathogen are mainly unknown. Here, we focused on the impact of glucose metabolism during pathogenesis. Glucose is highly abundant in the bloodstream and may also be available within tissues. In order to activate glucose to glucose-6-phosphate *A. fumigatus* possesses two catalytically active hexose kinases, glucokinase and hexokinase (Fleck and Brock, 2010). Analysis of deletion mutants and biochemical characterisations of the enzymes showed that glucokinase possesses a low K_m value for the substrate glucose and is required for rapid germination of conidia. In contrast, hexokinase possesses a broader specificity for various sugars and is the dominating hexose kinase during vegetative growth. Deletion of either one enzyme caused only weak effects on glucose, but the hexokinase was essential for growth on fructose. A mutant deleted in both genes revealed normal growth on most gluconeogenic nutrient sources, but growth was abolished when glucose or fructose served as sole carbon source. When tested in a murine model for invasive aspergillosis, both single mutants caused disease comparable to the wild type. However, the double deletion mutant was strongly attenuated in virulence regardless the immunosupression regimen. Thus, glucose seems to provide an abundant nutrient source during infection and interruption of glucose metabolism might provide a target to inhibit growth during pathogenesis. Fleck CB, Brock M. (2010) *Aspergillus fumigatus* catalytic gluco- and hexokinase: Expression analysis, importance for germination, growth and conidiation. *Eukaryot Cell* **9**:1120-35

371. Mechanisms of self resistance to zearalenone in *Fusarium graminearum*. Gerhard Adam[1], Juan Antonio Torres Acosta[1], Franz Berthiller[2], Gerlinde Wiesenberger[1], Rudolf Mitterbauer[1], and Rudolf Krska[2] [1]Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna (BOKU), 1160 Vienna, Austria. [2]Center for Analytical Chemistry, Department for Agrobiotechnology, IFA Tulln (BOKU), 3430 Tulln, Austria. gerhard.adam@boku.ac.at

The *Fusarium* mycotoxin zearalenone (ZON) is well known for its strong estrogenic activity in animals. Plants do not have an estrogen receptor, and it was unclear whether ZON has a biological role in plant-pathogen interaction. We have identified a prominent target for zearalenone: Hsp90. Zearalenone and more strongly beta-zearalenol (bZOL) inhibit ATPase activity of purified yeast Hsp90 (*Sc*Hsp82p) *in vitro*. Since ZON in high concentrations is also toxic for fungi, we have started to investigate mechanisms of self resistance. The ZON producer *F. graminearum* has a single gene encoding *HSP90*. Purified *Fg*Hsp90 is highly resistant to ZON inhibition. *In vitro* mutagenesis and generation of hybrid genes is used to identify the relevant residue implicated in increased target insensitivity. In addition, other mechanisms seem to be relevant. Upon challenge with ZON, *F. graminearum* rapidly detoxifies it to ZON-4-sulfate, which is no longer inhibitory in the Hsp90 ATPase assay. Funded by FWF SFB F3702 and F3706

372. Biosynthesis of the Cyclic Peptide Toxins of *Amanita* Mushrooms. Hong Luo, Heather E. Hallen-Adams, John S. Scott-Craig, and Jonathan D. Walton. DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824.

Fungi in the basidiomycetous genus *Amanita* owe their high mammalian toxicity to the bicyclic octapeptide amatoxins such as α-amanitin. Amatoxins and the related phallotoxins (such as the heptapeptide phalloidin) are encoded by members of the "MSDIN" gene family and are synthesized on ribosomes as short (34- to 35- amino-acid) proproteins based on an effective combinatorial strategy. Confocal microscopy has revealed the cellular and subcellular localizations of amanitin accumulation in basidiocarps (mushrooms) of *Amanita bisporigera*. Consistent with previous studies, amanitin is present throughout the basidiocarp (stipe, pileus, lamellae, trama, and universal veil), but it is present in only a subset of cells within these tissues. Several lines of evidence implicate a specific prolyl oligopeptidase, *A. bisporigera* POPB (AbPOPB), in the initial processing of the amanitin and phallotoxin proproteins. The gene for AbPOPB is restricted taxonomically to the amatoxin-producing species of *Amanita* and is clustered in the genome with at least one expressed member of the MSDIN gene family. Immunologically, amanitin and AbPOPB show a high degree of colocalization, indicating that toxin biosynthesis and accumulation occur in the same cells and possibly in the same subcellular compartments. Through next generation sequencing, other promising candidate genes for posttranslational modification have been found in the vicinity of the known toxin genes, and their analysis is in progress.

373. High throughput recombinant protein production of fungal secreted proteins. Andrea Vala[1], Doris Roth[1], Morten N. Grell[1] Anders Tunlid[2], and Lene Lange[1] [1] Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Copenhagen, Denmark [2] Department of Ecology, University of Lund, Sweden

Secreted proteins are important for both symbiotic and pathogenic interactions between fungi and their hosts. Our research group uses screens and genomic mining to discover novel proteins involved in these processes. To efficiently study the large number of candidate proteins, we are establishing a high-throughput protein production system with a special focus on fungal secreted proteins. We use a ligation independent cloning to clone target genes into expression vectors for *E. coli* and *P. pastoris* and a small scale test expression to identify constructs producing soluble protein. Expressed soluble proteins are then produced in larger quantities, purified and assayed for new enzymatic activities. We used transposon-assisted signal sequence trapping (TAST) to identify putative secreted proteins expressed during the interactions between the basidiomycete *Paxillus involutus* and birch (symbiotic interaction), between fungi of the order *Entomophthorales* and aphids (pathogenic interaction), and in the mycoparasitic interaction between the oomycetes *Pythium oligandrum* and *P. ultimum*. In general, the high-throughput protein production system can lead to a better understanding of fungal/host interactions and can also identify potential industrially useful enzymes.

374. Biodiversity discovery in Papua New Guinea: biological and chemical diversity of fungal endophytes. Johnathon Fankhauser[1], George D. Weiblen[1], Imke Schmitt[2], Daniel Ballhorn[1], Erin Treiber[1] & Georgiana May[1]. [1]Department of Plant Biology and Bell Museum of Natural History, University of Minnesota. [2]Goethe University Frankfurt and Biodiversity and Climate Research Centre BiK-F, Frankfurt, Germany.

We are taking a biotic inventory of fungal endophytes in a Papua New Guinea (PNG) fifty-hectare forest dynamics plot to assess phylogenetic diversity and potential pharmaceutical promise against malaria, cancer and HIV. We have isolated over two thousand endophytes from PNG trees. Forest dynamics plots provide opportunities for discovery of microbial diversity and infrastructure for bio-prospecting such as the ability to locate specific environments and host organisms. By sampling endophytes from mapped and identified trees in the permanent forest dynamics plot we have the opportunity to return to species or compound "rich" sampling sites to conduct more extensive sampling in the event of promising pharmaceutical leads. Here we evaluate fungal specificity with respect to host plant phylogeny of ten PNG trees and chemical diversity within unique fungal isolates. We are using phylogenetic approaches and Liquid Chromatography- Mass Spectrometry feature-detection metabolomics to develop a more directed sampling strategy for pharmaceutical drug discovery.

375. GlcNAc does not have to be catabolized to induce hyphal growth of *C. albicans*. Shamoon Naseem, Angelo Gunasekera, Esteban Araya, and James B. Konopka Stony Brook University; shamoonnaseem@hotmail.com

The amino sugar N-acetylglucosamine (GlcNAc) acts as both a structural component and a signaling molecule in a wide range of organisms from bacteria to humans. The mechanisms of GlcNAc signaling in fungi are not well understood, as the common lab yeasts *S. cerevisiae* and *S. pombe* do not grow on GlcNAc or respond to its presence. Therefore, we are studying the ability of GlcNAc to induce two pathways in the pathogenic fungus *Candida albicans*: one induces hyphal morphology and the other activates the genes needed to catabolize GlcNAc. We previously showed that transport of GlcNAc into the cell is needed for efficient signaling. To examine whether GlcNAc catabolism is important for signaling, we mutated the genes for the enzymes that phosphorylate (*HXK1*), deacetylate (*DAC1*), and deaminate (*NAG1*) GlcNAc to convert it to fructose-6-phosphate. Interestingly, the *hxk1*Δ mutant could be induced by GlcNAc to form hyphae. In contrast, GlcNAc was toxic to the *nag1*Δ and *dac1*Δ mutants. However, a triple *hxk1*Δ *dac1*Δ *nag1*Δ mutant could be induced to form hyphae, demonstrating that GlcNAc catabolism is not required to induce hyphae. This conclusion is supported by the fact that glucosamine does not induce hyphae. Experiments are underway to determine whether GlcNAc catabolism is needed to induce expression of the GlcNAc catabolic genes. Future studies will aim to define the mechanisms by which intracellular GlcNAc induces cellular signaling in *C. albicans*.

376. Biosynthetic Gene Cluster Dose Effect on Penicillin Production by *Penicillium chrysogenum*. Arnold J. M. Driessen 1, Jeroen G. Nijland 1, Stefan Weber 1, Marta Samol 1, Roel A. L. Bovenberg 2 1 Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG Groningen, Netherlands, and 2 DSM Anti-Infectives, A. Fleminglaan 1, 2611 XT Delft, Netherlands. E-mail: a.j.m.driessen@rug.nl

Industrial penicillin production levels by *Penicillium chrysogenum* have increased dramatically because of intense CSI. High-yielding strains contain multiple copies of the penicillin biosynthetic gene cluster that encodes three key enzymes of β-lactam biosynthesis. CSI also resulted in the up-regulation of precursor amino acid biosynthetic routes, peroxisomal biogenesis genes and of various transporters. This includes an ABC transporter that provides protection against the toxic effect of high levels of side chain precursor phenylacetic acid. We have analyzed the biosynthetic gene cluster dose effect on penicillin production using a high-yielding *P. chrysogenum* strain that was cured from its native clusters. The amount of penicillin V produced increased with the gene cluster number but saturated at high copy numbers. Transcription increased linearily with the cluster number. However, in contrast to the other biosynthetic enzymes, the protein level of acyltransferase, which localizes to peroxisomes, saturated already at low copy numbers. Finally, metabolome analysis supports the notion that the acyltransferase activity is limiting at high biosynthetic gene cluster copy numbers. Several approaches to alleviate this limitation will be discussed.

377. Genome-scale metabolic reconstruction and curation of the filamentous fungi *Neurospora crassa*. Jeremy Zucker1, Heather Hood2, Jonathan Dreyfus3, and James Galagan3. 1Broad Institute of MIT and Harvard, Cambridge MA 2OHSU, Portland, OR 3 Boston University, Boston, MA

We present NeurosporaCyc, a genome-scale flux balanced model of the filamentous fungi *Neurospora crassa* that is capable of representing, integrating, modeling and simulating the data avalanche of omics data being generated for this organism. We constructed this model using a combination of automated inference and manual curation based on the extensive literature for Neurospora. Furthermore, we have experimentally validated the growth phenotypes predicted by this model

378. Withdrawn

379. An active Fucosidase from *Fusarium oxysporum*. Janet Paper, John Scott-Craig, Mareike Bongers, and Jonathan Walton MSU/DOE Plant Research Laboratory, Michigan State University, East Lansing, MI paperjan@msu.edu

Alpha–fucosidase hydrolyzes fucose from alpha- fucosylated macromolecules. Due to the presence of fucosylated xyloglucan in plant cell walls, it also may be important for degradation of lignocellulosic biomass for biofuel production. Although fucosidase genes have been identified and characterized in *Arabidoposis*, rice, and many mammals, to our knowledge, no fungal fucosidase genes have yet been clearly identified; those listed in sequence databases are predictions based on homology. A protein with activity on 4-nitrophenyl-alpha-L-fucopyranoside had previously been identified and purified from *Fusarium oxysporum*; but, the encoding gene was apparently not identified (1). Using anion exchange and hydrophobic interaction chromatography, we have purified an alpha-fucosidase from the culture filtrate of *Fusarium oxysporum* strain 0-685 (isolated from cabbage) grown on fucose. The corresponding gene in the *Fusarium oxysporum f. sp. lycopersici* sequence database was identified by mass spectrometry-based proteomics. This protein is similar to enzymes in CAZy Glycosyl Family 29. Culture filtrates from *Trichoderma reesei*, *Fusarium graminearum*, and *Phanerochaete chrysosporium* grown on a variety of substrates including fucose did not secrete any detectable alpha-fucosidase. RACE experiments confirmed that the gene, FoFCO1, contains five introns which are also predicted in the sequence database of *Fusarium oxysporum f. sp. lycopersici*, but also contains an additional 66 nucleotides encoding 22 amino acids due to a size difference of one intron. BLAST alignments identify related predicted genes in some other fungi (e.g., *Leptosphaeria maculans* , *Magnaporthe oryzae* and *Cochliobolus heterostrophus*), but most fungi do not contain a clear ortholog. The gene is being expressed in *Pichia pastoris* to confirm its identity. Its range of activity on pNP- fucopyranoside as well as fucosylated plant xyloglucans will be discussed.
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380. Biochemical and biomechanical properties of the spore cell wall of Glomus irregulare. Pascal Arpin, Line Roy-Arcand, Anja Geitmann,Institut de Recherche en Biologie Végétale, Université de Montréal, Montréal, Québec , H1X 2B2, Canada, pascal.arpin@umontreal.ca

Mycorrhizal fungi are increasingly used for augmenting the yield in agricultural applications. Although fungal spores are known to be resistant structures, they are subjected to stress-inducing or mechanically damaging conditions during the manufacturing process for agricultural use. Some conditions could affect the viability of the spores and thus the efficiency of the product. In order to investigate the relationship between spore robustness in Glomus irregulare and the preservation of their ability to colonize plants under various field conditions we assessed their structure and mechanical behavior using a wide array of techniques:1) micro-indentation to determine spore stiffness and visco-elasticity, 2) scanning electron microscopy to analyse the effects of different matrices/conditions on the wall morphology and 3) confocal laser scanning microscopy in combination with histochemistry and immunodetection to determine wall composition. Chitin and glomalin are major constituents of the fungal cell wall. We assessed the distribution and relative abundance of these components based on label with monoclonal antibodies and with lectins that binds specifically with N- acetylglucosamine residues. Since G. irregulare produces intraradical and extraradical propagules, we compared the data obtained for each type depending on their origin and their age. This is the first attempt to relate fungal spore mechanics with the biochemical composition of the cell wall.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

381. Structure-activity relationships of antifungal plant defensins MsDef1 and MtDef4. Uma Shankar Sagaram, Raghoottama Pandurangi, Jagdeep Kaur, Dilip Shah. Donald Danforth Plant Science Center, 975 N Warson Rd., St. Louis, MO. USA

Plant defensins are small cysteine-rich antimicrobial proteins (AMPs) stabilized by four disulfide bonds. MsDef1 and MtDef4 from *Medicago* spp. inhibit the growth of a filamentous fungus *Fusarium graminearum* at micromolar concentrations but use different modes of action. MsDef1 is a morphogenic defensin inducing hyperbranching of fungal hyphae, whereas MtDef4 is a nonmorphogenic defensin that does not cause obvious morphological alterations. We have determined structure-activity relationships of these two defensins and found that each defensin contains a highly conserved three-dimensional gamma-core motif ($GXCX_{3-9}C$), a hallmark feature of AMPs with disulfide bonds. The gamma-core motif of each defensin was shown to be the major determinant of antifungal activity and the induction of hyperbranching phenotype. Replacement of the gamma-core motif of MsDef1 with that of MtDef4 resulted in a significant increase in the antifungal activity and converted MsDef1 to a nonmorphogenic defensin. The sequence motifs present in the loop region of gamma-core motif that are important for the *in vitro* antifungal activity of MsDef1 and MtDef4 were also characterized. Furthermore, the antifungal activity of defensins was not solely dependent on the rate of fungal plasma membrane permeabilization. Also, the analysis of peptides corresponding to the gamma core-motif of MtDef4 indicated that cationic and hydrophobic amino acids are important for antifungal activity.

382. Regulation of the Activity and Cellular Localization of the Circadian Clock Protein FREQUENCY. Joonseok Cha, Haiyan Yuan and Yi Liu. Department of Physiology, The University of Texas Southwestern Medical Center, Dallas, TX.

Eukaryotic circadian clocks consist of autoregulatory circadian negative feedback loops. In the filamentous fungus *Neurospora*, FREQUENCY (FRQ), the FRQ-interacting RNA helicase FRH, and two transcriptional factors WHITE COLLAR-1 (WC-1) and WC-2 are the core components in the circadian negative feedback loop. To close the transcription-based negative feedback loop, the FRQ-FRH complex inhibits the activity of the WC complex in the nucleus. Despite its essential role in the nucleus, most of the FRQ protein is found in the cytoplasm. It was previously proposed that the phosphorylation of FRQ regulates its cellular localization. In this study, we mapped the FRQ regions that are important for its cellular localization. We showed that the C-terminal part of FRQ, especially the FRQ-FRH interaction domain, plays a major role in controlling FRQ localization. Both the mutations of the FRQ-FRH interaction domain and the downregulation of FRQ result in the nuclear enrichment of FRQ, suggesting that FRH regulates FRQ localization through its interaction with FRQ. To study the role FRQ phosphorylation, we examined the FRQ nuclear/cytoplasmic localization at different circadian times and in array of FRQ kinase, FRQ phosphatase and FRQ phosphorylation sites mutants. Collectively, our results strongly suggest that FRQ phosphorylation does not play a significant role in regulating its cellular localization. Instead, we found that phosphorylation of FRQ inhibits its transcriptional repressor activity due to its influence on the FRQ-WC interaction. Out results suggest that the rhythmic FRQ phosphorylation profile during a circadian cycle is an important part of the mechanism that drives robust circadian gene expression.

383. Transcriptome analysis of the ectomycorrhizal fungus *Paxillus involutus* provide insights into the mechanisms of organic matter conversion. Francois Rineau[1],Firoz Shah[1], Tomas Johansson[1], Björn Canbäck[1], Lene Lange[2], Doris Roth[2], Mark Smits[3],Per Persson[4], Erika Lindquist[5], Igor V. Grigoriev [5], Anders Tunlid[1] [1]Department of Biology, Lund University, Sweden; [2]Copenhagen Institute of Technology/Aalborg University, Section for Sustainable Biotechnology, Denmark; [3] Hasselt University, Belgium; [4]Department of Chemistry, Umeå University, Sweden; [5]US DOE Joint Genome Institute, Walnut Creek, CA, USA.

We have examined how the ECM fungus *P. involutus* degrade soil organic matter (SOM) using elemental analysis, FTIR, pyrolysis-GC/MS, and synchronous fluorescence. In parallel, the pattern of gene expression was analyzed using 454 EST sequencing. The chemical analysis showed that the fungus were degraded and converted plant cell wall polysaccharides, aromatic compounds (phenolics), proteins and heterocyclic N-related compounds. Analysis of ca 200,000 EST sequences showed that *P. involutus* had a limited set of genes encoding for enzymes involved in the coversion of plant cell wall polysaccharides and phenolics. Our data suggest that the conversion of SOM by *P. involutus* it least partly is due to a non-enzymatic degradation, i.e. free radical generation driven by Fenton chemistry, making its degrading system similar to those identified in brown rot fungi.

384. Withdrawn

385. Histone deacetylase inhibitor HC-toxin from *Alternaria jesenskae*. Wanessa D. Wight[1,2] and Jonathan D. Walton[1,2]. [1] Department of Energy - Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, U.S.A.[2] Cell and Molecular Biology Program, Michigan State University, East Lansing, MI 48824, U.S.A. wightwan@msu.edu

HC-toxin is a cyclic tetrapeptide with known histone deacetylase (HDAC) inhibition activity. It is an essential virulence factor for the maize pathogen *Cochliobolus carbonum*. Biosynthesis of HC-toxin is controlled by a complex genetic locus, *TOX2*, that spans >500 kb. *TOX2* contains at least seven genes including the four-domain nonribosomal peptide synthetase, HTS1. All of the TOX genes are present in two to three copies at the *TOX2* locus. *Alternaria jesenskae* (1), also produces HC-toxin (R. Labuda, personal communication). A genome survey sequence of *A. jesenskae* was generated by 454 pyrosequencing. Unambiguous orthologs of all seven known genes involved in HC-toxin biosynthesis from *C. carbonum* were identified in *A. jesenskae*. The average percent identities of the TOX genes from the two fungi range from 80 to 85%. As in *C. carbonum*, many of the HC-toxin genes are present in two copies in *A. jesenskae*. Variation in the genomic organization of the TOX genes in addition to the high degree of similarity among housekeeping genes suggest that the HC-toxin clusters in the two fungi might be the result of evolution from a common ancestor and not the result of horizontal gene transfer event. 1.Labuda R., Eliás P. Jr., Sert H., Sterflinger K. 2008. *Alternaria jesenskae* sp. nov., a new species from Slovakia on *Fumana procumbens* (Cistaceae). Microbiol Res.163(2):208-14

386. Assessment of biosynthesis of 2-pentylfuran by *Aspergillus* spp. Shrawan Bhandari[1], Richard Weld[2], Amy Scott-Thomas[1], Jon Palmer[3], Nancy Keller[3], Steve Chambers[1]. [1]University of Otago, New Zealand. [2]Lincoln Ventures, New Zealand. [3]University of Wisconsin.

Aspergillus spp. are filamentous fungi that can cause Invasive Aspergillosis (IA) in the immunocompromised population. During growth, these fungi release a volatile organic compound (VOC), 2-pentyl furan (2-PF), which has been reported to be a potential biomarker for the diagnosis of IA by breath analysis. Linoleic acid is the precursor of 2-PF and biosynthesis of this VOC in *Aspergillus* spp. is believed to occur via the action of lipoxygenase (*lox*) and/or dioxygenase (*ppo*) enzymes. The aim of this study was to determine whether 2-PF is a metabolic product of *Aspergillus* spp. and whether it is synthesized via the *lox* and/or *ppo* pathway. *Lox* and/or *ppo* knock-out and knock-down strains of *A. fumigatus, A. nidulans* and *A. flavus* were compared with respective wild-type strains for 2-PF production. These strains were grown in air-tight culture vials and analysis of the head-space gas for 2-PF was performed using Gas Chromatography-Mass Spectrometry (GC-MS/MS). The levels of 2- PF produced by these mutants of *A. fumigatus* (P= A. nidulans (P=0.001) were significantly lower than the wild-type strain. It appears that *lox* and/or *ppo* genes are involved in the pathway of 2-PF biosynthesis in these fungi, however, neither of these genes seems to be involved exclusively in the biosynthesis of 2-PF.

387. Targeted gene deletion and genome mining of the predicted *Aspergillus carbonarius* secondary metabolome. Michael Praseuth[1], Antonia Gallo[2], Scott E. Baker[3] , Clay C. C. Wang[1], and Kenneth S. Bruno[3] [1]Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angeles, California, USA. [3]Chemical and Biological Process Development Group, Energy and Environment Directorate, Pacific Northwest National Laboratory, Richland, Washington, USA [2]National Research Council, Institute of Science of Food Production (ISPA) Bari, Italy

Interest in natural products produced by fungi has grown substantially in recent years. Available genome sequences of filamentous fungi have revealed that these organisms contain the capacity to produce far more secondary metabolites than previously identified by conventional analysis. In large part, the reason that these compounds remain uncharacterized is due to the lack of gene expression under typical laboratory conditions. One approach to identifying novel compounds produced by fungi is heterologous expression of predicted secondary metabolite genes in a tractable model organism such as *Aspergillus nidulans*. Although this has proved successful with some compounds, an analysis in the native organism could prove more fruitful in determining all of the enzymes necessary to produce a given compound. For this reason we have developed a transformation protocol for transformation in Aspergillus carbonarious strain ITEM 5010. We have successfully deleted the kusA orthologue in this strain using the hygromycin phosphotransferase marker. In a subsequent transformation we deleted the orthologue of an Aspergillus niger gene responsible for naptha-pyrone production. Gene deletion and overexpression will be used to identify genes responsible for production of secondary metabolites in this species.

388. Heterotrimeric G-alpha protein Pga1 from *Penicillium chrysogenum* triggers germination in response to carbon sources through a cAMP-mediated pathway, and affects negatively resistance to stress conditions. Francisco Fierro[1,2], Ramón O. García-Rico[1,3], Francisco J. Fernández-Perrino[2], Juan F. Martín[1] [1]Instituto de Biotecnología de León, INBIOTEC, España [2]Depto. de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, México DF, fierrof@xanum.uam.mx [3]Departmento de Microbiologia, Universidad de Pamplona, Colombia

We studied the role of Pga1 in spore germination and resistance to stress conditions in *Penicillium chrysogenum*. Strains G203R-T (expressing the dominant inactivating *pga1*^{G203R} allele) and D*pga1* (deleted *pga1*) showed a delayed and asynchronic germination pattern, and a decrease in the percentage of germination, which occurred in only 70-80% of the total conidia. In contrast, in strains expressing the dominant activating *pga1*^{G42R} allele, germination occurred at earlier times and in 100% of conidia. In addition, strains with the *pga1*^{G42R} allele were able to bypass the carbon source requirement for germination in about 64% of conidia. Thus Pga1 plays an important, but not essential, role in germination, mediating carbon source sensing. Regulation of germination by Pga1 is probably mediated by cAMP, as intracellular levels of this secondary messenger undergo a peak before the onset of germination only in strains with an active Pga1. Pga1 activity is also a determinant factor in the resistance to stress conditions. Absence or inactivation of Pga1 allow growth on SDS-containing minimal medium, increase resistance of conidia to thermal and oxidative stress, and increase resistance of vegetative mycelium to thermal and osmotic stress. In contrast, constitutive activation of Pga1 causes a decrease in the resistance of conidia to thermal stress and of vegetative mycelium to thermal and osmotic stress. Together with our previously reported results, we conclude that Pga1 plays a central role in the regulation of the whole growth-developmental program of this biotechnologically important fungus.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Population and Evolutionary Genetics

389. "Magic traits" drive the emergence of pathogens. Pierre Gladieux[a,b], Fabien Guérin[b], Tatiana Giraud[a], Valérie Caffier[b], Christophe Lemaire[b], Luciana Parisi[b], Frédérique Didelot[b], Bruno Le Cam[b]. [a]Univ. Paris Sud/ CNRS, UMR Ecologie Systématique Evolution, Orsay, France; [b]INRA/Univ. Angers, UMR PaVé, Beaucouzé, France. E-mail: pierre.gladieux@u-psud.fr

An important branch of evolutionary biology strives to understand how divergent selection for an ecologically important trait can foster the emergence of new species specialized on different niches. Such ecological speciation is usually difficult to achieve because recombination between different subsets of a population that are adapting to different environments counteracts selection for locally adapted gene combinations. Traits pleiotropically controlling adaptation to different environments and reproductive isolation are therefore the most favourable for ecological speciation, and are thus called "magic traits". We used genetic markers and cross-inoculations to show that pathogenicity-related loci are responsible for both host adaptation and reproductive isolation in emerging populations of *Venturia inaequalis*, the fungus causing apple scab disease. Because the fungus mates within its host and because the pathogenicity-related loci prevent infection of the non-host trees, host adaptation pleiotropically maintains genetic differentiation and adaptive allelic combinations between sympatric populations specific to different apple varieties. Such "magic traits" are likely frequent in fungal pathogens, and likely drive the emergence of new diseases.

390. The utility of Genome Survey Sequences in developing anonymous loci for population-level studies in Oomycetes. Jaime E. Blair, Andrew G. Staffaroni, and Amy M. Chabitnoy. Department of Biology, Franklin & Marshall College, Lancaster PA

Genome Survey Sequences (GSS) are random clone libraries that represent a "snapshot" of an organism's genome. GSS libraries have several advantages over traditional cDNA libraries; sheared genomic DNA is used as the source material (as opposed to mRNA), so no special conditions are needed to obtain high quality nucleic acids. GSS data also contain both coding and non-coding sequences, which allows for the identification of simple sequence repeats (i.e., microsatellites) and other anonymous loci that are important for species- and population-level studies. In this project, we have generated GSS libraries for several Oomycete species, primarily *Phytophthora*. Genomic DNA was extracted from individual isolates, sheared, cloned into a standard vector, and sequenced. Traditional Sanger sequencing has produced approximately 1.4 million basepairs of data from approximately 1200 clones, although Next-Generation sequencing technologies are being evaluated. In an analysis of three floral pathogens (*P. cactorum, P. cryptogea*, and *P. nicotianae*), GSS sequences were used to identify potential diagnostic and phylogenetic loci. We are also developing GSS libraries to assist in Oomycete diversity surveys in local Pennsylvania waterways.

391. Global Population Structure and Aflatoxin Chemotype Diversity in *Aspergillus* Section *Flavi*. Geromy G. Moore[1], Bruce W. Horn[2], Jacalyn L. Elliott[1], Alex R. Griffing[3], Eric A. Stone[3], Kerstin Hell[4], Sofia N. Chulze[5], German Barros[5], Graeme Wright[6], Manjunath K. Naik[7], Ignazio Carbone[1] [1]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695 [2]National Peanut Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Dawson, GA 39842 [3]Department of Bioinformatics, North Carolina State University, Raleigh, NC 27695 [4]International Institute of Tropical Agriculture, Cotonou, Republic of Benin [5]Departamento de Microbiologia e Immunologia, Universidad Nacional de Rio Cuarto, Córdoba, Argentina [6]Department of Primary Industries, Queensland, Kingaroy, Australia [7]Department of Plant Pathology, College of Agriculture, Karnataka, India

Aspergillus section *Flavi* includes species known to produce carcinogenic polyketides known as aflatoxins. *A. flavus* and *A. parasiticus* are the most common agents of aflatoxin (AF) contamination of important agricultural commodities worldwide. With recent discoveries of sexual stages in *A. flavus* and *A. parasiticus*, it is possible that sexual recombination may increase genetic and AF chemotype diversity in populations. To determine the impact of sex on AF chemotype diversity we examined molecular sequence variation within the AF gene clusters of *A. flavus* and *A. parasiticus* from geographically isolated fields in Africa (Benin), Argentina, Australia, United States (Georgia), and India. We found that that the ratio of mating-type genes in populations of *A. flavus* and *A. parasiticus* correlated with the amount of linkage disequilibrium in the AF cluster and with chemotype diversity. Significant differences in the frequencies of mating-type genes indicate a clonal population structure, which in *A. flavus* results in a unimodal distribution favoring nonaflatoxigenicity and in *A. parasiticus* results in AF concentrations following a single binomial distribution with B1- and G1-dominant peaks. In sexual *A. flavus* populations there is a significant shift to aflatoxigenicity, and in *A. parasiticus* sex reduces the frequency of B1- and G1-dominant chemotypes such that population G1/B1 ratios follow a unimodal normal distribution. Worldwide phylogeographic and population genetic analyses were performed with six species in section *Flavi* to test for population subdivision and gene flow potential. There was no geographic differentiation within species based on variation at four non-cluster loci (*amdS*, *MAT*, *mfs* and *trpC*); however there was evidence of functional (chemotype) divergence at two cluster loci (*aflM/aflN* and *aflW/aflX*). *Aspergillus flavus* S_{BG} strains in Benin showed the greatest genetic heterogeneity in a combined principal component analysis with cluster and non-cluster loci.

392. Molecular phylogenetics in the anthracnose pathogen *Colletotrichum acutatum sensu lato*
Riccardo Baroncelli[1], Charles Lane[2], Ulrike Damm[3], Paul Cannon[4] , Giovanni Vannacci[5], S. Sreenivasaprasad[6] and Dez Barbara[1]. [1] School of Life Sciences, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK. [2] FERA, Sand Hutton, York, YO41 1LZ, UK. [3] CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. [4] CABI Europe-UK, Bakeham Lane, Egham, Surrey TW20 9TY, UK. [5] Dept Tree Science Entomology and Plant Pathology, University of Pisa, Via del Borghetto 80, 56100 Pisa, Italy. [6] Division of Sciences, University of Bedfordshire, Park square, Luton, YLU1 3JU, UK

Colletotrichum acutatum is an important pathogen causing economically significant losses of temperate, subtropical and tropical crops. Globally, *C. acutatum* populations display considerable genotypic and phenotypic diversity. The overall objective is to understand the molecular relationships within the species with particular reference to the pathogen populations associated with strawberry production systems in the UK.
More than 200 *C. acutatum* isolates isolated from different hosts worldwide have been assembled. Phylogenetic analysis of sequence data from the rRNA gene block-ITS region, GPDH and the beta-tubulin 2 gene led to the identification of distinct genetic groups within *C. acutatum*. The sets of isolates within these genetic groups corresponded to the groups A1 – A9 identified previously on the basis of rRNA ITS sequences. Almost all of the isolates capable of homothallic sexual reproduction, both in culture and in nature, comprise a single genetic group. Information regarding isolates representing

populations capable of heterothallic sexual reproduction suggests they belong to two genetic groups A3 and A5. Molecular characterization of *C. acutatum* populations representing the introduction and spread of the pathogen into strawberry production systems in the UK showed the presence of at least three genetic groups A2, A3 and A4. Overall, our results suggest the existence of *C. acutatum* populations potentially undergoing speciation processes, related to their reproductive behavior and host association patterns. Further molecular and phenotypic characterization is in progress.

393. Monitoring Oomycete species diversity in a local Pennsylvania watershed. Amy Chabitnoy, Lauren Cook, and Jaime E. Blair Department of Biology, Franklin & Marshall College, Lancaster PA

Species of the genera *Phytophthora* and *Pythium* (Oomycota) are well known plant pathogens. These organisms infect a diverse array of plants and are responsible for causing devastating problems in agricultural and commercial industries as well as native habitats. Oomycete species possess certain characteristics that aid in pathogenicity and invasiveness, such as their ability to survive in moist, humid environments and their propensity to be introduced to new locations via commodity exchange. In this project, we are sampling the diversity of Oomycete species present in a local cold water stream in southcentral PA; this waterway serves as a model for the larger Chesapeake Bay watershed as the surrounding vegetation is representative of the area. Samples have been collected using water filtration methods, rhododendron leaf baiting, hemp seed baiting, and intact pear fruit baiting of both water and soil samples. DNA was extracted from each isolate and identified to species via sequencing and phylogenetic analysis of the large ribosomal RNA subunit (LSU) locus. One species, *Phytophthora gonapodyides* was present in abundance regardless of sampling strategy or season. To further explore the role of this species in the environment, we are generating a Genome Survey Sequence (GSS) library. The GSS data will be compared to the available genomic sequences of other Oomycetes in order to create a genomic snapshot of this organism.

394. Unusual features of the Botrytis cinerea mating system. Jan A.L. van Kan (1), Paul S. Dyer (2) and Linda M. Kohn (3) 1 Laboratory of Phytopathology, Wageningen University, The Netherlands, 2 School of Biology, University of Nottingham, United Kingdom; 3 Department of Ecology and Evolutionary Biology, University of Toronto, Canada. E-mail: jan.vankan@wur.nl .

Botrytis cinerea is a heterothallic ascomycete with two mating types, MAT1-1 and MAT1-2. Fragments of the MAT1- 2-1 and MAT1-1-1 genes were detected bordering idiomorphs of MAT1-1 and MAT1-2 isolates, respectively. Both these fragments encode truncated, non-functional proteins. *B. cinerea* has probably evolved from a homothallic ancestor containing all genes, with MAT1-1 and MAT1-2 arising from the loss of HMG and alpha-domain sequences, leaving the disabled gene fragments present in current loci. Two ORFs, designated MAT1-1-5 and MAT1-2-3, have not previously been reported from other fungi. In a cross of a MAT1-1-5 knockout mutant with a wild type MAT1-2 strain, the stipe develops normally but transition to the differentiation of a cup is blocked. Most *B. cinerea* isolates act in a standard heterothallic fashion, but some isolates can mate with both MAT1-1 and MAT1-2 isolates and are referred to as 'dual maters'. Some dual mater isolates can self-fertilize and are truly homothallic. The MAT locus of five dual mater isolates was analysed. Four of those contain a MAT1-2 locus, without any part of the MAT1-1 locus being detected, whereas one homothallic isolate contains a MAT1-1 locus, without any part of the MAT1-2 locus being detected. We conclude that dual mating and homothallism are controlled by factors other than the MAT locus.

395. Species-specific expansion of Chitin binding CBM18 domain in the amphibian killing *Batrachochytrium dendrobatidis*: a role in virulence? John Abramyan and Jason E Stajich. University of California, Riverside, CA. abramyan@ucr.edu

Batrachochytrium dendrobatidis (Bd) is the only known amphibian pathogen in the Chytridiomycota. Despite being such a specialized pathogen from a generally non-pathogenic lineage, Bd is exceptionally virulent and is thought to have a large role in amphibian decline and extinction worldwide. In this study, we analyzed the genome sequence of Bd to characterize the expansion of Carbohydrate-Binding Module Family 18 (CBM18); a domain which confers binding properties, potentially critical for pathogenesis. CBM18 was found in 67 copies, across 18 putative loci (in comparison, *Spizellomyces punctatus* has only three domain copies). Phylogenetic analysis of the domains revealed considerable diversification, but also well supported clades grouped by gene locus, suggesting functional specialization. Anywhere from 1 to 11 tandem duplicates of domains were found per locus. While some loci contained multiple copies of domains from various phylogenetic groups, other domain groups were found within a single locus. Diversified CBMs in a locus may signal specificity to a multivalent substrate. Conversely, the expansion of a domain group in a locus may confer stronger binding power. In addition to CBM18, select loci also contain tyrosinase and deacetylase domains, both of which have been associated with pathogenesis in fungi. Our results suggest that we have identified a group of genes which have undergone a Bd specific expansion likely associated with the evolution of its pathogenic lifestyle.

396. Tracing the origin of the fungal alpha1 domain places its ancestor in the HMG-box superfamily. Tom, Martin [1]#, Shun-Wen, Lu [2]#, Herman, van Tilbeurgh [3], Daniel, R. Ripoll [2], Christina, Dixelius[1], B. Gillian, Turgeon [2], Robert, Debuchy [3] [1]SLU, Uppsala, Sweden [2]Cornell University, Ithaca, USA [3]Univ Paris-Sud, Orsay, France # Equal contribution

Fungal mating types in self-incompatible Pezizomycotina are specified by one of two alternate sequences occupying the same locus on corresponding chromosomes. One sequence is characterized by a gene encoding an HMG protein, while the other, a gene encoding a protein with an alpha1 domain. DNA-binding HMG proteins are well characterized. In contrast, alpha1 domain proteins evolutionary origin is obscure, precluding a complete understanding of mating-type evolution in Ascomycota. alpha1 proteins have not yet been placed in any of the large families of sequence-specific DNA-binding proteins. We present sequence comparisons, phylogenetic analyses, and *in silico* predictions of secondary and tertiary structures, which support our hypothesis that the alpha1 domain is related to the HMG domain. We have also characterized a new conserved motif in alpha1 proteins of Pezizomycotina. This motif is downstream of the alpha1 domain and consists of a core sequence Y-[LMIF]-x(3)-G-[WL] in a larger conserved motif. Our data suggest that extant alpha1-box genes originated from an ancestral HMG gene, which confirms the current model of mating-type evolution within the fungal kingdom. We propose to incorporate alpha1 proteins in a new subclass of HMG proteins termed MATalpha_HMG.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

397. Closely related species of *Phaeosphaeria* infecting wheat at the host center of origin. Megan C. McDonald, Patrick C. Brunner and Bruce A. McDonald Plant Pathology, Institute of Integrative Biology, ETH-Zuerich, Switzerland.

The genetic relationships among *Phaeosphaeria* species that infect wheat and other cereals are not well understood. *Phaeosphaeria nodorum*, the most widely recognized wheat pathogen within the genus, is composed of two genetically distinct biotypes on wheat and barley. A lesser-known species, *Phaeosphaeria avenaria* exists as two formae species, *Phaeosphaeria avenaria avenaria* (Paa), on oats and *Phaeosphaeria avenaria tritici* (Pat) on wheat and barley. Pat has been further divided into Pat1, Pat2 and Pat3, based on RFLP fingerprints. This study expanded on earlier work using sequence data to define species infecting wheat in a global sample. We sequenced 4 loci, a total of 2,221 bp, in 309 global isolates, including a large number from the host center of origin, Iran. We were able to identify *P. nodorum*, Pat1 and Pat3 in addition to two previously unknown groups, tentatively named P1 and P2. *P. nodorum* was the largest group, followed by Pat1. Only 6 Pat3 isolates were identified in a Danish population. P1 and P2 were found only in Iran. We analyzed the phylogenetic relationship among these sister species and used coalescent gene-trees to reconstruct the mutational history of each locus. Based on our analyses, we believe these 5 groups to represent the major wheat-infecting clades of *Phaeosphaeria*. Coalescent analysis suggests that P1 and P2 are the most recent common ancestors of the globally distributed *P. nodorum*.

398. Back to the future – sexuality of the human pathogen *Aspergillus fumigatus.* Edyta Szewczyk, Fabian Schröter, and Sven Krappmann Research Center for Infectious Diseases, Julius-Maximilians-University Würzburg, Würzburg, Germany

Sexual reproduction of the human pathogen *Aspergillus fumigatus* (teleomorph: *Neosartorya fumigata*) was assumed to be absent or cryptic until recently, when fertile crosses among geographically restricted environmental isolates were described. The existence of cryptic sexuality in this species had been proposed long before, based on genomic and genetic analyses revealing presence of the mating type idiomorphs (*MAT1-1* and *MAT1-2*) and several putative genes orthologous to recognized determinants of pheromone signalling, mating, karyogamy, meiosis and fruiting body formation in the fertile species *Aspergillus nidulans*. Furthermore, the products of *A. fumigatus MAT1-2* and *MAT1-2* genes were shown to be functional in *A. nidulans*. We provide evidence for mating, fruiting body development, and ascosporogenesis accompanied by genetic recombination between unrelated clinical isolates of *A. fumigatus*, which demonstrates the generality and reproducibility of this long-time undisclosed phase in the lifecycle of this heterothallic fungus. Furthermore, we demonstrate that successful mating requires the presence of both mating-type idiomorphs *MAT1-1* and *MAT1-2*, as does expression of genes encoding factors presumably involved in this process. Given the narrow conditions that favor sexual development in *A. fumigatus* accompanied by the strict need for the opposite mating type, we were furthermore interested in the creation of a presumed homothallic strain expressing both mating-type idiomorphs and therefore being capable of progression through the initial stages of sexual development and fruiting body formation. Detailed inspection and phenotypic characterization of such a self-fertile isolate will yield insight on the evolutionary origin of homothallism and might also provide information on the role of restricted sexuality for fungal virulence.

399. Withdrawn

400. Rapid and Diversified Molecular Evolution of the *AvrLm7* Avirulence Gene of *Leptosphaeria maculans* under *R* Gene Selection in the Field. G. Daverdin[1], L. Gout[1,2], T. Rouxel[1] and M.H. Balesdent[1] [1] INRA-Bioger, Grignon, France ; [2] AgroParisTech, Grignon, France

Leptosphaeria maculans, the agent of the stem canker of oilseed rape, develops "gene-for-gene" interactions with its host plant, and shows a high evolutionary potential to "breakdown" novel resistance sources in the field. The newly marketed *Rlm7* resistance allowed us to monitor the evolution of populations faced to this resistance over three consecutive years and to identify molecular events responsible for the loss of the corresponding *AvrLm7* avirulence. Population genetics approaches were also used to evaluate the main population traits linked with birth and dissemination of the mutations in the populations. A total of 1530 isolates was collected over the three years in a single field, on one cv. harbouring *Rlm7* and one cv. devoid of it. During this period *avrLm7* virulent isolates collected on the susceptible cv. increased in frequency from 10% to nearly 40%, but remained undetectable in neighbouring fields. All possible types of molecular events leading to virulence were found, with an over-representation of complete deletions and wide-scale RIP mutations of the coding sequence. Our study points out the importance of mutability of genes involved in the interaction, directly linked with their genome environment, and reproduction/mating system rather than geographical dissemination, which appear to be a minor threat for the resistance breakdown in the *L. maculans-B. napus* pathosystem.

401. Adaptive divergence of an effector of the apple scab in relation to domestication of apple tree. Christophe, Lemaire, Céline, Rousseau, Thibault, Leroy, Amandine, Lê Van, Aiguo, Qin, Pierre, Gladieux, and Bruno, Le Cam ECOFUN, UMR PAVE 77 INRA- AgroCampus Ouest-Université d'Angers

Domestication has a great impact on evolution of host-parasite interactions. In fact for a pathogen host domestication can be considered as the raise of a new potential host. Then survival on newly domesticated host is probably linked to adaptive changes, especially on loci directly involved in interaction with host. *Venturia inaequalis* is the pathogen agent of apple scab. Origin of this fungus has been previously located in Central Asia where it infects the ancestor of the domesticated apple tree. In order to better understand the adaptive changes in *V. inaequalis* during domestication process, we analyzed the molecular variation of several genes involved in interaction with apple tree. Sampling was particularly focused on Central Asia natural ecosystems (ancestral) and agrosystem (domesticated). Polymorphism of an effector Vice16, divided samples into two divergent clades : «ancestral» *vs* «post-domestication». Tests for adaptive divergence permitted to reject neutral evolution at this locus. We also found occurrence of a selective sweep at the base of the «domestic» clade. Hitchhicking at the 3' flanking region was evidenced. Hitchhicking slope analysis permitted to infer migration/selection parameters on Vice16. This study is then one of the first reference for adaptive change of a pathogen linked to domestication of his host. This study was granted by ANR Emerfundis and Fungisochore

402. Emergence and global invasion of the apple scab fungus *Venturia inaequalis* in connection with the domestication of its host. Bruno Le Cam [1], Pierre Gladieux[1], Valérie Caffier[1], Thibault Leroy[1], Xiu Guo Zhang[2], Isabelle Roldan Ruiz[3], Els Coart[3], Rosa Maria Valdebenito Sanhueza[4] and Mohamed Sbaghi[5]. [1] INRA, UMR077 PAVE, 49071 Beaucouze, France. lecam@angers.inra.fr [2] Department of Plant Pathology, Shandong Agricultural University, Taian, China. [3] ILVO, Plant Growth and Development, Caritas straat 21, 9090 Melle, Belgium. [4] EMBRAPA, Bento Gonçalves, Brazil. [5] INRA, Kenitra, Morocco.

Venturia inaequalis is an ascomycete responsible for apple scab, a disease that has invaded all apple growing regions worldwide. It also infects wild species of Malus, and in particular the Central Asian Malus sieversii that is the main progenitor of the domesticated apple, and the European crabapple M. sylvestris. As the origin and expansion history of this fungus were unknown, we analyzed the population genetic structure of V. inaequalis from samples collected in apple orchards from the five continents using microsatellite variation. Comparison of the levels of variability among populations, along with coalescent analyses of migration models and estimates of genetic distances, was consistent with a scenario in which the fungus emerged in Central Asia before its introduction into Europe that plays a role of bridgehead in the colonization of the disease into other continents with the expansion of apple growing. In order to test the hypothesis of a host tracking scenario where the fungus followed its host during the domestication process, we analyzed the genetic structure of populations collected from wild Malus and domesticated apples in Central Asia and Europe. We identified three distinct populations: (i) a large European population on domesticated and wild apples, (ii) a large Central Asian population on domesticated and wild apples in urban and agricultural areas, and (iii) a more geographically restricted population in M. sieversii forests growing in the eastern mountains of Kazakhstan. Unique allele richness and divergence time estimates supported a host-tracking scenario in which this latter population represents a relict of the ancestral populations from which current populations found in human-managed habitats were derived. This work was funded by a Programme de Recherches Avancees managed by the Association Franco-Chinoise pour la Recherche Scientifique et Technique, by the Région Pays de la Loire, INRA and an ANR grant 07-BDIV-003 Emerfundis.

403. Retracing the History of World-Wide Invasions of the Oilseed Rape Pathogen *Leptosphaeria maculans.* A. Dilmaghani[1], L. Gout[1,2], P. Gladieux[3], T. Giraud[3], P. Brunner[4], M.H. Balesdent[1] and T. Rouxel[1] [1] INRA-Bioger, Thiverval-Grignon, France [2] AgroParisTech,Thiverval-Grignon, France [3] ESE, Université Paris-Sud, Orsay, France [4] ETH Zurich, Switzerland

This work aimed at retracing the history of the invasions in several regions of the world of the fungal plant pathogen *Leptosphaeria maculans*, causing stem canker (Blackleg), an economically important disease of oilseed and vegetable Brassicas, and to assess whether introduced populations acquired specific features. The worldwide population structure, genetic diversity, mode of reproduction and migration patterns of the fungal pathogen were analyzed using 14 minisatellite markers, designed from the whole-genome sequence. The populations of *L. maculans* exhibited high levels of genetic diversity and a low degree of genetic structure in Europe, USA, eastern Canada and western Australia. A lower genetic diversity was found in populations from western Canada and Chile, with a high clonal fraction, an unusual feature for *L. maculans* populations. These latter populations appeared both highly differentiated one from each other and from other populations in the world. The introduction in western Canada was therefore associated with a strong bottleneck and the acquisition of specific features. Patterns of allelic richness, genetic distances and isolation-with- migration models were consistent with a European origin of the pathogen and inferred dates of introduction into different regions of the world were recent (between 190 and 390 years), suggesting a link with the recent introductions of Brassicas as food and oil sources in these parts of the world.

404. Withdrawn

405. Genetic and phenotypic diversity of *Sclerotinia sclerotiorum* on a small geographic scale. Renuka N. Attanayake[1], Lyndon Porter [2], Dennis Johnson[1], and Weidong Chen [3]. [1]Department of Plant Pathology, Washington State University (WSU), Pullman, WA. [2] USDA- ARS, Prosser, WA. [3]USDA- ARS, and WSU, Pullman, WA.

Population structure, genetic and phenotypic diversity of *Sclerotinia sclerotiorum* were investigated on a small geographic scale. A collection of 40 *Sclerotinia* isolates from one square meter top layer of soil in a Washington alfalfa field was studied for colony color, Mycelial Compatibility Groupings (MCGs), oxalic acid production, DNA haplotypes of eight microsatellite loci, fungicide sensitivity and virulence. The 40 isolates exhibited three colony colors: beige (22), dark (11) and white (7). Fifteen MCGs and 16 microsatellite haplotypes were found among the 40 isolates. There were several examples that isolates within a MCG belonged to different haplotypes and that isolates of the same haplotype belonged to different MCGs. STRUCTURE analyses indicated there were five clusters, suggesting likely five genetic populations. All isolates showed considerable oxalic acid production except that one isolate consistently produced significantly less amount of acid, on a pH-indicating medium. The isolates also exhibited significant differences in sensitivity to fungicides benomyl, fluzinam and iprodione, and in virulence as measured by colonization on detached pea leaves. This study documents high level of genetic and phenotypic diversity of *S. sclerotiorum* on a small geographic scale, presenting challenges in managing the diseases it causes.

406. Distribution and evolution of fusarin mycotoxin biosynthetic genes in *Fusarium*. Maria Teresa Amatulli[1,2], Chris Maragos[1], Mark Busman[1], Maria Lodovica Gullino[2], Robert H. Proctor[1] [1]US Department of Agriculture, ARS, NCAUR, Peoria, Illinois, USA; [2]Agroinnova, University of Turin, Grugliasco, Torino, Italy

In *Fusarium/Gibberella*, secondary metabolite biosynthetic (SMB) genes that have a narrow distribution within the genus can have complex evolutionary histories. Whether more widely distributed SMB genes have similarly complex histories is not known. Genes responsible for production of fusarin mycotoxins may provide an opportunity to address this question because they occur in at least two distantly related species, *F. verticillioides* and *F. graminearum*. The fusarin polyketide synthase gene (*FUS1*) and eight genes (*FUS2 - FUS9*) immediately upstream of it are thought to be a fusarin biosynthetic gene cluster based on their co-expression under culture conditions conducive to toxin production. Here, we examined genetically diverse fusaria by Southern and PCR analysis for the presence of *FUS* genes. We detected the genes in multiple species of three *Fusarium* lineages: the *Gibberella fujikuroi* species complex (GFSC), the *F. tricinctum* species complex, and the trichothecene- producing clade. In contrast, we did not detect *FUS* genes in the *F. oxysporum* species complex or in closely related species (e.g. *F. miscanthi*), nor did we observe them in the genome sequence database for *F. oxysporum f. sp. lycopersici*. Preliminary analyses indicate that *FUS* gene-based phylogenies are concordant with primary metabolic gene-based phylogenies for most but not all fusaria. Our data indicate that *FUS* genes are widely distributed but not present uniformly in *Fusarium* and that their evolutionary histories can be complex.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

407. Evolution of a new plant pathogen by hybridization. Inderbitzin Patrik, R. Michael Davis, Richard M. Bostock and Krishna V. Subbarao. Department of Plant Pathology, University of California at Davis, One Shields Avenue, Davis, CA 95691.

Hybridization plays a central role in plant evolution, but its overall importance in fungi is unknown. It has been suggested that new plant pathogens could arise by hybridization between formerly separated fungal species. Evolution of hybrid plant pathogens from non-pathogenic ancestors in the fungal-like protist *Phytophthora* has been demonstrated, but in fungi, the most important group of plant pathogens, there are few well-characterized examples of hybrids. We focused our attention on the diploid hybrid and plant pathogen *Verticillium longisporum*, the causal agent of the Verticillium wilt disease in crucifer crops. In order to address questions related to the evolutionary origin of *V. longisporum*, we used phylogenetic analyses of seven nuclear loci and a dataset of 203 isolates of *V. longisporum*, *V. dahliae* and related species. We confirmed that *V. longisporum* was diploid, and originated three different times, involving four different lineages and three different parental species. All hybrids shared a common parent, species A1 that hybridized respectively with species D1, *V. dahliae* lineage D2 and *V. dahliae* lineage D3, to give rise to three different lineages of *V. longisporum*. Species A1 and species D1 constituted as yet unknown taxa. *Verticillium longisporum* likely originated recently, as each *V. longisporum* lineage was genetically homogenous, and comprised species A1 alleles that were identical across lineages. There is evidence that the three different hybrid lineages differ in virulence. Financial support provided by the California Leafy Greens Board, USDA-NIFA, and the University of California Agricultural Experiment Station.

408. *Botrytis pseudocinerea*, a new cryptic species causing grey mould in French vineyards in sympatry with *Botrytis cinerea*. Anne-Sophie Walker* (1), Angélique Gautier (1), Johann Confais (1), Muriel Viaud (1), Pascal Lepêcheur (1), Joelle Dupont (2), Elisabeth Fournier (3) (1) UR BIOGER-CPP, Avenue Lucien Brétignières, F78850 Thiverval-Grignon (2) MNHN, 57 rue Cuvier, F75008 Paris (3) UMR BGPI, TA A 54/K, Campus International de Baillarguet, F34398 Montpellier *walker@versailles.inra.fr

Botrytis cinerea, the anamorph of *Botryotinia fuckeliana*, is an ubiquitous fungus causing gray mold on many crops, including vegetables, ornamental plants and fruits and especially grapevine. This disease was recently found to be caused by a complex of two related fungal species living in sympatry: *Botrytis* group II (=*B. cinerea sensu stricto*), the most frequent species in the complex, and Botrytis group I (=*B. pseudocinerea* as proposed name), which is present in weak abundance in French vineyards. Here we compared morphological and biological criteria, as well as pathogeny on tomato and green bean between the two species, without finding any significant difference. Nevertheless, isolates from group I were found to be naturally resistant to the fungicide fenhexamid in comparison to group II wild-type strains. Multiple-gene phylogeny confirmed that the two groups clearly cluster into two fully supported monophyletic clades, and unambiguously established the phylogenetic position of the phylogenetic species *B. pseudocinerea* into the Botrytis genus, previously revised. The transposable element *Flipper*, formerly used to characterize *B. cinerea* populations, was proved to be a non- diagnostic marker to recognize these cryptic species. Population analyses using microsatellite markers confirmed the lack of gene flow between the two species, and enabled to assess respective genetic diversity in each species and to pinpoint private diagnostic alleles for *Botrytis* group I. At last, no viable progeny was produced from crosses between group I and group II strains. As biological, ecological, phylogenetic and genetic criteria confirmed the distinction between the two groups, we conclude that *Botrytis* group I is clearly a distinct species that occupies similar ecological niches as *B. cinerea sensu stricto* and may contribute, to a lesser extend, to grey mould epidemics in French vineyards.

409. Sex determination in the original sexual fungus. Alexander Idnurm. School of Biological Sciences, University of Missouri-Kansas City, 5100 Rockhill Road, Kansas City MO 64110, USA

The first report of sex in the fungi dates two centuries ago to the species *Syzygites megalocarpus* (Mucormycotina). This organism was used by Blakeslee as a representative of self-fertile species, leading to the development of the concepts of heterothallism and homothallism for the kingdom. Here, two putative *sex*/*MAT* loci were identified in a single strain of *S. megalocarpus*, revealing the basis for homothallism. The species encodes copies of both of the HMG-domain containing SexM and SexP proteins, flanking by conserved RNA helicase and glutathione oxidoreductase genes found adjacent to the mating type loci in other Mucormycotina species. The presence of pseudogenes and the arrangement of genes suggests the origin of homothallism in this species from a heterothallic relative via chromosomal rearrangements to bring together both loci into a single genetic background.

410. Differentiation of *Fusarium graminearum* and *F. asiaticum* by using novel microsatellite markers. Seung-Ho Lee, Young Ju Nam, Seung Wan Son, Soohyung Lee, Mija Kim, Jong-Chul Yun, Jae-Gee Ryu, Theresa Lee Microbial Safety Division, NAAS-RDA, Suwon, 441-707, Korea

Recent studies revealed that trichothecene producing *Fusarium* species are distributed with geographical preference. In Asia, *F. asiaticum* is especially dominant in cereals including rice and barley. To study genetic diversity between *F. graminearum* and *F. asiaticum* and also within each species, we tested Korean *Fusarium* populations with previously known microsatellite markers such as F1, F11, HK-917, and MS-Fg103 first. F1 and F11 markers appeared to be useful to differentiate *F. graminearum* and *F. asiaticum*. Sequence analysis of the above microsatellites from *F. graminearum* and *F. asiaticum* strains (two of each) showed lack of repeats in *F. asiaticum* strains in general. To explore genetic diversity at novel microsatellite loci, we developed 33 new markers after identifying repeat sequences in the genome of *F. graminearum* with a SciRoKo program. Based on the PCR band patterns by electrophoresis, these novel microsatellite primers could be classified into five distinct groups showing variation between two species. Further analysis of polymorphism at these loci is in progress.

411. The 5' untranslated region plays an important role in the expression of the *nsdC* gene which encodes a positive regulator of sexual development of *Aspergillus nidulans*. Lee-Han Kim and Dong-Min Han Division of Life Sciences, Wonkwang University, Korea

The *nsdC* gene encodes a C2H2 type transcription factor that regulates sexual development positively and also asexual development negatively in *Aspergillus nidulans*. The NsdC is predicted to consist of 643 amino acids with a C2H2 C2H2 C2HC type zinc finger DNA binding domain in the middle of the polypeptide. The *nsdC* gene generates two distinct transcripts, 3.0 and 2.6 kb. The 5'UTR of larger transcript reaches near 1.0 kb including two relatively long introns (168 bp and 212 bp), while the 2.6 kb transcript lacks the most part of 5'UTR. Differential accumulation of the 2.6 kb transcript in various stages of growth and development as well as under the varying environmental conditions indicates that the expression of *nsdC* is subject to a complex post transcriptional regulation in which 5'-UTR plays an important role. Hence, the role of various region of 5'UTR was examined by analyzing the effects on the expression using GUS reporter and the phenotypic changes, including carbon source dependent retarded vegetative growth and hyper-active asexual development observed in the *nsdC* deletion mutant. The result showed that the upstream region of 5'UTR including first intron functioned as promoter but its downstream region could not.

412. The evolution of pheromonal signal system in homothallic *Neurospora*. Rebecka Strandberg[1], Kristiina Nygren[1], Magnus Karlsson[2], Anastasia Gioti[1], and Hanna Johannesson[1] [1]Department of Evolutionary Biology, Uppsala University, Uppsala, Sweden. [2]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden. Rebecka.Strandberg@ebc.uu.se

The genus *Neurospora* is ideal for studies on reproductive system evolution, since it contains taxa with different types of reproductive systems, *i.e.* heterothallism, homothallism, and pseudohomothallism. In heterothallic *Neurospora*, sexual reproduction is only possible for strains of opposite mating type (*mat a* and *mat A*). The *mat* genes govern the whole expression cascade of mating-type-specific expressed genes, including pheromone receptor genes (*pre-1* and *pre-2*) and pheromone precursor genes (*ccg-4* and *mfa- 1*). In homothallic taxa, all information crucial for sexual reproduction reside in one haploid nucleus. Earlier, signs of genetic decay of the *mat* genes in homothallic *Neurospora* have been described. Here, we study the molecular evolution of the *pre*-genes in homothallic taxa, to see if they show signs of genetic decay or are conserved. Preliminary analyses suggest that the *pre* genes, in contrast to the *mat* genes, are under the same evolutionary constraints in heterothallic and homothallic taxa. To investigate if the genes are regulated and spliced in a similar fashion regardless of reproductive strategy, we study the expression levels and cDNA-splicing of pheromone receptor and pheromone precursor genes in homothallic *Neurospora*.

413. Multilocus analyses of the citrus brown spot pathogen *Alternaria alternata* suggest multiple phylogenetic lineages and recombination. Jane E. Stewart[1], Kalyn A. Thomas[1], Chris B. Lawrence[2], Tobin L. Peever[1] [1]Dept of Plant Pathology, Washington State University, Pullman, WA [2]Virginia Bioinformatics Institute, Blacksburg, VA

Alternaria species are thought to be asexual based on the absence of described teleomorphs. Molecular evolutionary analyses of *Alternaria* mating type genes show that these loci are under purifying selection suggesting a role in recombination. The objective of this study was to examine the mating system of *Alternaria alternata*. Isolates of *A. alternata* causing citrus brown spot were sampled from a citrus grove in Florida. Isolate mating type was determined and isolates were sequenced at six loci. Loci were selected as physically unlinked on 6 large scaffolds of the *A. brassicicola* genome assembly. Three populations were identified. Both mating types were found in populations 1 and 3 with more *MAT1-1* in population 1 (92%) and more *MAT1-2* in population 3 (74%). Gametic disequilibrium was detected in populations 1 and 3, supporting a clonal mating system. However, recombination events were localized to isolates of opposite mating type. In population 2, reticulation was found in network analyses, and we were unable to reject a random mating model. This suggests putative mitotic recombination, since all isolates were *MAT1-2*. Results demonstrate that *A. alternata* infecting citrus is predominately asexual but has the ability to recombine through sexual/asexual means.

414. Characterization of evolutionary phenomena through serial passage experiment in the rice blast fungus, *Magnaporthe oryzae*. Junhyun Jeon[1,2], Jongsun Park[1], Ralph A. Dean[2], Yong-Hwan Lee[1] [1]Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea [2]Functional Genomics, North Carolina State University, Raleigh, North Carolina, USA

Little is known about evolutionary phenomena in plant pathogenic fungi that results from repeated in-vitro subculture despite its obvious implications on experimental design and interpretation of data. Here, we followed the virulence of three independent reference strain (70-15) cultures of the rice blast fungus *Magnaporthe oryzae* during 20 generations of growth in a serial passage experiment on oatmeal agar media. Three independent lineages showed no significant differences in growth rate but considerable variations in colony morphology and pigmentation within- and between lineages after 10 generations. Some but not all lineages started to show significant reduction in asexual sporulation and/or appressorium formation at about generation 15. Virulence tended to decrease in all three lineages, though to varying extent, as subculturing continued. Interestingly, fungal cultures isolated from lesions caused by generation 20 cultures showed lower virulence than the founding culture, although they tended to be more virulent than the cultures from which they were derived. In an attempt to understand the genetic basis for the differences between founding and terminal generation 20 cultures, we examined DNA fingerprinting patterns of cultures using a dispersed repeated DNA sequence, MGR586, and DNA methylation patterns based on the use of isoschizomers. However, we found no clear differences among cultures. We are also employing next-generation sequencing technology to examine genome changes with precision during our serial passage experiment. We expect our efforts would provide insights into stability of pathogenicity and genome evolution in plant pathogenic fungi.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

415. The role of somatic recombination in natural populations of the root pathogen *Armillaria mellea*. Kendra Baumgartner and Phillip Fujiyoshi. USDA-ARS, Dept. of Plant Pathology, Univ. of California, One Shields Avenue, Davis, CA 95616, USA, kbaumgartner@ucdavis.edu .

Fungi have evolved various mechanisms of shuffling genetic material, which can occur in the absence of fruiting and meiosis. In the homobasidiomycete *Armillaria* (causal agent of Armillaria root disease), the predominant vegetative stage is diploid. Diploid and haploid mycelia can fuse and undergo karyogamy to create a recombinant diploid mycelium, although the mechanism by which this occurs is not known. Nonetheless, evidence of somatic recombination in natural populations of *Armillaria* suggests that this process may be important in the pathogen's ecology. Within the genus, *A. mellea* in particular has a very broad host range (500+ plant species) and among different hosts it exhibits different types of symbioses (pathogen, saprophyte, or mycorrhiza). Such characteristics require phenotypic plasticity. Somatic recombination in *A. mellea* may facilitate genetic adaptation, as the diploid genome is thought to be particularly resistant to mutation, genotypes are long-lived, fruiting is seasonal and the pathogen does not form asexual spores. Our goal is to evaluate the role of somatic recombination in adaptation of *A. mellea*. As a first step, we mated diploid and haploid mycelia, and are characterizing the stability of the synthesized mycelia. In matings between a wild-type diploid and transgenic, hygromycin-resistant haploids, we identified recombinant, hygromycin-resistant diploids, and additionally, hygromycin-resistant triploids. Genotype and uninucleate status of each recombinant diploid and hybrid triploid strain were found to be stable in serial transfers carried out over a 4-mo. period (to date), replicated with and without selection. Greater fitness of diploid and especially triploid strains, relative to haploid strains, suggests that ploidy influences fitness in *A. mellea*. On-going steps in the experiment are to determine the influence of environmental stress on fitness and stability of the synthesized mycelia, and to assess the fertility of the triploid strains.

416. Discovery of a sexually reproducing population of the main fungal pathogen of rice, Magnaporthe oryzae, in Asia. D. Tharreau*, D. Saleh, E. Fournier, P. Xu, C. Li, H. Adreit, J. Milazzo, V. Ravigné, E. Bazin, D. Tao & J.-L. Notteghem. * UMR BGPI, TA A54/K, Baillarguet Campus, 34000 Montpellier, France. didier.tharrea@cirad.fr

Determining if recombination occurs is of uttermost importance, particularly in pathogenic species, since it impacts the adaptive potential of populations. This might be strenuous in fungi, where sex can be cryptic or facultative. Magnaporthe oryzae is the heterothallic fungus responsible for rice blast. Its sexual cycle was never observed in the field and the worldwide population genetic structure is consistent with asexual reproduction. However, previous scattered data suggested that recombination could occur in limited areas of South Asia. Here we provide biological and population genetics evidences that M. oryzae reproduces sexually in some localities in China. In one population, almost all strains were female fertile, whereas this phenotype required for sexual reproduction is rare or absent in most populations. Strains from complementary mating types were also present in equal frequencies and underwent normal sexual cycle in vitro. Population genetics parameters fitted a recombinant population. Computer simulations confirmed that this genetic structure could not be observed without recombination. An in vitro evolution experiment showed the loss of female fertility after few clonal reproduction cycles. The wild phenotype was not restored by various stresses. The loss of female fertility segregated in progenies of crosses between evolved strains and wild ones, indicating a putative genetic basis.

417. Origins of self-compatibility: A comparative study including 4 new *Neurospora* genomes. Anastasia Gioti, Alexandra Mushegian, Eugenie Pessia, Hanna Johannesson. Evolutionary Biology EBC, Uppsala University.

The fungal genus *Neurospora* comprises species showing a wide range of mating systems, from self-incompatibility (heterothallism) to self-compatibility (homothallism) and pseudohomothallism, an intermediate between the two. The ancestral mating system is unknown for this genus and remains controversial in fungi. Inter-species comparisons of synteny along the *mat* locus, containing the genes controlling sexual reproduction in fungi, were proven a powerful means to elucidate this question[1-2] in other genera. In *Neurospora*, the genomes of two heterothallic and one pseudohomothallic species of the same phylogenetic clade[3] are available. Homothallic taxa are numerous, but with the exception of a few published gene sequences[4-6], they are practically unstudied. We sequenced and *de novo* assembled the genomes of 4 homothallic species and experimentally confirmed their *mat* locus structure. Here we present a comparative study of the mating type locus within the genus. We identified two distinct mechanisms potentially involved in the evolution of self-compatibility: Unequal cross-over events and a transposable element - mediated translocation. A model proposing multiple transitions to homothallism from a heterothallic ancestor will be discussed. 1. Galagan 2005. Nature 438. 2. Butler 2009. Nature 459. 3. Nygren 2011. Mol. Phyl. Evol., accepted. 4. Pöggeler 1999. Curr. Genet. 36. 5. Dettman 2001. Fungal Genet. Biol. 34. 6. Wik 2008. BMC Evol. Biol. 8.

418. Somatic fusion in fungi as a model for the evolution of cooperation. Eric Bastiaans, Fons Debets and Duur Aanen Wageningen University, Wageningen, The Netherlands

Cooperative behaviors, behaviors that benefit other individuals, are widespread. However, to understand cooperation we have to explain how cheating, i.e. profiting without contributing, is kept at low frequency. Kin-selection is the predominant solution for this problem. Kin selection requires that cooperation is preferentially directed towards related individuals, and one way to achieve this is through genetic kin recognition. However, Crozier argued that in the short term positive frequency dependent selection will eliminate the genetic polymorphism required for such recognition, since common genotypes will experience more cooperation and thereby increase in frequency. Here we study somatic fusion between mycelia of the ascomycete *Neurospora crassa* as a potential model for cooperation. We first show that fusion between genetically identical mycelia is net beneficial, and thus cooperative. We then show experimental evidence for Crozier's theoretical prediction that, in the short term, positive frequency dependent selection acts against polymorphism of recognition genes (i.e. heterokaryon incompatibility genes). With these findings we discuss which counteracting evolutionary forces maintain the extensive recognition polymorphism that restricts somatic fusion in fungi.

419. Understanding the parasexual gene transfer system between the mucoralean mycoparasite *Parasitella parasitica* and its host *Absidia glauca.* Burmester, A., Karimi, S., Wetzel, J., Wostemeyer, J. Chair of General Microbiology and Microbe Genetics, FSU Jena, Neugasse 24, 07743 Jena, Germany

During infection, the mycoparasite *Parasitella parasitica* forms a cytoplasmic continuum with its hosts, leading to the transfer of nuclei and other organelles. Due to the anatomy of infection structures, these transfer events occur unidirectionally from the parasite to the host. This interaction leads finally to uptake of the parasite's DNA into host nuclei [1]. Using *Absidia glauca* as host, we demonstrated the transfer of artificial plasmids and of several chromosomally encoded biosynthesis genes. Recently, we especially analyzed the transfer events between an adenine-auxotrophic *P. parasitica* donor mutant and a methionine-auxotrophic mutant of *Absidia glauca*. We characterized the biosynthetic block of the Met$^-$-mutant to reside in the gene for homoserine-acetyl-transferase. We also identified two genes for the corresponding biosynthetic function in the methionine auxotrophic donor by cloning and sequencing. Based on these prerequisites, we are now able to follow the fate of donor DNA in mycelia and nuclei of *A. glauca* recipients by PCR and hybridization approaches. [1] Wostemeyer *et al.* (2002) in: Horizontal gene transfer (eds: Syvanen, Kado) 21, pp 241-247.

420. Reverse Transcription of the pFOXC Mitochondrial Retroplasmids of *Fusarium oxysporum* is Protein- Primed. Jeffrey Galligan, Haedar Abuirqeba and John Kennell, Saint Louis University, St. Louis, MO

The pFOXC retroplasmids are linear, autonomously-replicating genetic elements that reside in mitochondria of certain strains of *Fusarium oxysporum.* They have a unique clothespin structure that includes a 5'-linked protein and telomere-like terminal repeats, with pFOXC1 and pFOXC3 having multiple copies of a 3 bp and 5 bp sequence, respectively. The plasmids have a single ORF that encodes a reverse transcriptase (RT) that was previously shown to use both RNA and DNA primers to initiate reverse transcription. Here, we provide evidence that the RT uses a protein to prime reverse transcription, making it only the second RT reported to employ a protein primer. Reverse transcription reactions having endogenous or exogenous RNA templates generate cDNA products that are covalently attached to a protein. When *in vitro* reactions are carried out with a single radiolabeled dNTP, a labeled protein is produced that migrates at the size of the pFOXC-RT, suggesting that the primer is the RT itself. Reactions with pFOXC1-RT using a full complement of dNTPs undergo a deoxynucleotidylation step prior to elongation and show a nucleotide specificity that corresponds with sequences of the terminal repeat, while deoxynucleotidylation associated with pFOXC3-RT exhibits a different nucleotide preference that partially matches the 3' terminal repeat. Based on RT phylogeny and the multiple mechanisms of cDNA initiation, our studies suggest that the pFOXC plasmids could be progenitors to a wide range of retroelements, including the telomerase complex.

421. Dispersal processes underlying the recent pandemic caused by the plant pathogenic fungus *Mycosphaerella fijiensis.* J. Carlier*, S. Robert*, A. Rieux*, F. Halkett†, M-f. Zapater*, L. De Lapeyre De Bellaire‡, C. Abadie*and V. Ravigne* * CIRAD, UMR BGPI, Campus international de Baillarguet, TA A-54K, F-34398 Montpellier Cedex 5, France. † Present adress ; INRA, Nancy-Université, UMR 1136 Interactions Arbre-Microorganismes, F-54280 Champenoux, France. ‡ CIRAD, Persyst, UPR Syst. Banan. Ananas, TA B-26 / PS4, Blvd. de la Lironde, 34398 Montpellier Cedex 5, France

How plant pathogenic fungi spread is the first question to consider for understanding the emergence of diseases caused by such organisms. *Mycosphaerella fijiensis* causing the black leaf streak disease of banana is an example of a recent pandemic in agriculture and a good model to address this question in the case of an aerial plant pathogen. The pandemic started around 1960 from the South-East Asia. Samples from various populations around the world at different geographical scales were analyzed using nuclear sequence-based and microsatellite markers. Demographic events (founder effects or admixture) were detected at global and continental scales following introductions of the disease. These introductions were more likely due to movement of infected plant materials. At lower scale, the structure of the *M. fijiensis* populations in two recently (~1979-1980) colonised areas in Costa Rica and Cameroon was analysed. Genetic differentiation and isolation by distance (IBD) were detected in both countries along a ~250-300km-long transect, suggesting continuous range expansion through gradual dispersal of spores over a few hundred kilometres. Furthermore, a discontinuity in gene frequencies was observed along the Cameroon transect. A landscape genetic study was recently conducted around this discontinuity. No landscape features matched the genetic discontinuity supporting it could result from a demographic event during the spread of *M.fijiensis* in the country rather than a physical barrier impeding contemporary gene flow. The genetic structure observed in *M. fijiensis* populations at different geographical scales has allowed a better understanding of dispersal processes in such an organism.

422. Evidence for natural hybridization among homothallic members of the basidiomycete *Armillaria mellea*. Sarah E. Bergemann[1], Bethany R. Baker[1], and Kendra Baumgartner[2]. [1]Middle Tennessee State Univ., Biology Dept., PO Box 60, Murfreesboro, TN 37132, USA, sbergema@mtsu.edu [2]USDA-ARS, Dept. of Plant Pathology, Univ. of California, One Shields Avenue, Davis, CA 95616, USA, kbaumgartner@ucdavis.edu .

Populations of *Armillaria mellea* (Basidiomycota, Agaricales, Physalacriaceae) are typically heterothallic; homothallic populations are reported only from Africa (*A. mellea ssp. africana*), China [*Armillaria* China Biological Species (CBS) G], and Japan (*A. mellea ssp. nipponica*). Monosporous isolates of heterothallic strains are haploid, and their mating behavior is consistent with that of a tetrapolar mating system. In contrast, monosporous isolates of homothallic strains, which are the equivalent of diploid zygotes, bypass the haploid mycelial phase by packaging diploid nuclei into single spores (homoheteromixis). Because *A. mellea* is a virulent pathogen of many crops, the genetic origins of this switch from selfing to outcrossing or vice versa is of concern with respect to disease spread. In an effort to understand the origins of homothallism among members of*A. mellea* from different geographic regions, we constructed mutational networks and conducted independent phylogenetic analyses of multiple, nuclear protein-coding genes. Genealogical discordance among genes was evident from the analyses of multiple, unlinked loci, although a polyphyletic origin of homothallism in *A. mellea* was clear based on the separation of homothallic members into multiple clades. Homothallic *Armillaria* CBS G collapsed into a single clade and shared identical haplotypes with heterothallic strains only from China. In homothallic isolates of *A. mellea* from Africa and Japan, haplotypes from diploid isolates separated into two clades along with heterothallic members from populations originating from China and Europe. This pattern clear of separation of haplotypes from a single isolate is indicative of hybridization, with putative origins from Europe and China. Whether homothallism is the ancestral state is unclear, as genealogical discordance posed potential problems with identifying the ancestral clade, and remains the focus of future investigations using coalescent approaches.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

423. Inferring evolutionary relationships of *Phytophthora* species in the Ic clade using nuclear and mitochondrial genes. Erica Lasstier [1], Carsten Russ[2], Chas Nusbaum[2], Qiandong Zheng[2], Chia-Hui Hu[1], Jeff Thorne[1], and Jean Ristaino[1]. [1] NC State University, Raleigh, NC and [2] The Broad Institute, MIT, Cambridge, MA.

Phytophthora infestans , the causative agent of potato and tomato late blight is an important pathogen worldwide and caused the Irish potato famine of the 1840's. Two sisters species of *P. infestans* in the Ic clade, *P. andina* and *P. mirabilis* have been described in Ecuador and Mexico, respectively. We sequenced the mitochondrial genomes of the sister species of *P. infestans* in the Ic clade and used the whole genome sequences and coalescent analysis to resolve the evolutionary histories of members of the Ic clade. Both gene order and content were conserved among the Ic clade *Phytophthora* species. The rooted tree inferred documents the earliest divergence separating *P. phaseoli* from the rest of the Ic clade species. Next, the lineage ending at *P. andina* diverged from the lineage leading to *P. infestans*. *P. mirabilis* and *P. ipomoeae* diverged more recently from a *P. andina* ancestor. The inferred tree shows that *P. mirabilis* and *P. ipomoeae* are more closely related to each other than either is to *P. infestans*. Our data are consistent with the hypothesis that the Andean region is the center of evolutionary origin for all the species in the clade since *P. andina* and *P. infestans* coexist there and bridging hosts occur there. Further surveys are needed to test this hypothesis and the occurrence of *P. mirabilis* and *P. ipomoeae* in the Andean region.

424. Testing the Novel versus Endemic Pathogen Hypotheses for presence of *Batrachochytrium dendrobatidis* in British Columbia. Jonathon LeBlanc, Dr. Will Hintz, Dr. Brad Anholt Department of Biology University of Victoria PO Box 3020, Station CSC Victoria, BC V8W 3N5 Canada, jleblanc@uvic.ca

A recently discovered amphibian pathogen, *Batrachochytrium dendrobatidis* (*Bd*), has been found to infect and cause mortality in many different amphibian species. In the last few years *Bd* has been detected in local frog populations on Vancouver Island. It is not known whether this is a local strain of the *Bd* or a new strain that was recently introduced to the area. The emergence of new infectious diseases has been explained by two different hypotheses. The novel pathogen hypothesis states that the pathogen has just recently been introduced into the area and is being vectored by a newly introduced host. Preliminary data suggest that the vector for transmission of *Bd* is the frog species *Rana catesbeiana*. The alternate hypothesis suggests that the pathogen is endemic and is only now becoming pathogenic due to changes in biotic or abiotic factors in the environment. These factors can either act to lower the immune response of the amphibians or act on the pathogen resulting in a more virulent strain. One of the best ways to test these alternate hypotheses is to compare populations of the pathogen, which are found with and without the known vector, from a variety of amphibian species on Vancouver Island and Mainland British Columbia.

425. Microsatellite composition across five *Fusarium* species. Santana QC*, Coetzee MPA*, Steenkamp ET°, Wingfield MJ* and Wingfield BD*.*Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. °Department of Microbiology and Plant Pathology, FABI, University of Pretoria, South Africa

Microsatellites are commonly used for population studies due to their informative length polymorphisms. However, in fungi the identification of these markers is difficult due to their relatively low abundance. The aim of this study was to use a genomic approach to study the frequency and similarity of microsatellites between various species of *Fusarium*. For this purpose we searched the genomes of five species: *F. graminearum, F. verticillioides, F. oxysporum fsp. lycopersici, F. solani* and *F. circinatum* for microsatellites repeat motifs, after which the microsatellite flanking regions were analysed based on sequence similarity. The number of identified microsatellites ranged between 2202 and 4539 between the species. Of these, the di-nucleotides were the predominant repeats, whereas hexanucleotide motifs had the lowest frequency. Analyses of the flanking regions showed that 14.2% of the microsatellites among the species represent 860 groups, with flanks above 95% similarity. Microsatellites that grouped based on flanking region similarity among the species should be targeted for cross species amplification, while the remaining microsatellites could represent good candidates for developing species-specific markers. Such genomic approaches would therefore be invaluable for developing markers to be used in both diagnostic and population biology studies of fungi.

426. Unorthodox gene structure and mobile elements in mitochondrial DNA of Glomeromycota. Maryam Nadimi[1], Denis Beaudet[1], Lise Forget[2], Franz Lang[2] and Mohamed Hijri[1]. [1] Université de Montréal, Département de sciences biologiques, Institut de recherche en biologie végétale (IRBV), 4101 rue Sherbrooke Est, Montreal, QC, H1X 2B2, Canada. [2] Robert Cedergren Centre for Bioinformatics and Genomics, Département de Biochimie, Université de Montréal, 2900 Boulevard Edouard-Montpetit, Montréal, Québec, H3T 1J4, Canada

Arbuscular mycorrhizal fungi (AMF) are a group of obligatory plant symbionts with coenocytic, multigenomic hyphae. AMF mtDNA is otherwise peculiar in gene structure, harboring a multitude of introns and related mobile elements. In *Glomus diaphanum* we found four distinct partial gene duplications, separated by ORFs encoding homing endonucleases which is the result of a transposition event mediated by an endonuclease. The presence of gene duplication may result in erroneous interpretation of PCR and sequencing results in unknown species, but with more complete sequences available, may also be turned into an opportunity for designing specific molecular markers. Moreover in the mtDNA of *Gigaspora rosea*, we found that the *cox1* gene occurs in two pieces, separated by about 30kbp of coding sequence. We show at the RNA level, that the corresponding *cox1* exons are joined by a rare groupI intron- mediated trans-splicing process. Thus, we have the opportunity to identify Glomeromycota at species and isolate level, as well as recognizing the donor(s) of the partial gene duplications and the evolution of trans-splicing.

427. Global molecular surveillance provides a framework for understanding diversity within the *Fusarium graminearum* species complex. Todd J. Ward[1], Kerry O'Donnell[1], Diego Sampietro[2], Anne-Laure Boutigny[3],and Altus Viljoen[3] [1] USDA-ARS, [2] Universidad Nacional de Tucumán, [3] University of Stellenbosch

Members of the *Fusarium graminearum* species complex (FGSC) are responsible for diseases of a variety of cereal crops worldwide. These species also are a significant food-safety concern because they contaminate grain with trichothecene mycotoxins that inhibit eukaryotic protein synthesis and can modulate immune function. In order to establish a global picture of FGSC diversity, a recently developed multilocus genotyping assay was used to assess species and trichothecene chemotype diversity among a world-wide collection of more than 8,000 FGSC isolates. The results revealed: 1) substantial regional variation in species and trichothecene chemotype composition and diversity, 2) recent changes in diversity and composition due to transcontinental movement, and 3) evidence of species-specific differences in host preference. In addition, the global population structure of *F. graminearum* was analyzed using variable number tandem repeat markers. Significant population differentiation was observed within this mycotoxigenic cereal pathogen, and evidence of the recent transcontinental movement of populations followed by limited genetic exchange between resident and introduced populations was uncovered.

428. Evolutionary relationships among Aspergillus flavus vegetative compatibility groups. Lisa C. Grubisha United States Department of Agriculture, Agricultural Research Service, Tucson, AZ 85721 Lisa.Grubisha@ars.usda.gov Peter J. Cotty United States Department of Agriculture, Agricultural Research Service, School of Plant Sciences, University of Arizona, Tucson, AZ 85721 pjcotty@email.arizona.edu

Aspergillus flavus is a fungal plant pathogen with diverse plant hosts including cotton, peanuts, maize, almond, and pistachio. During infection by *A. flavus*, crops are frequently contaminated with aflatoxins, highly carcinogenic mycotoxins. *A. flavus* populations are composed of numerous vegetative compatibility groups (VCGs), however not all VCGs produce aflatoxin. Crosses between *A. flavus* VCGs with different mating type loci have been induced in laboratory studies. We have developed a set of 24 microsatellite markers for *A. flavus* from across the eight chromosomes to investigate genetic diversity, reproductive mode and population structure. We examined the genetic structure of *A. flavus* populations, including a study of YV36, the VCG to which the biocontrol fungus AF36 belongs. Results from these studies demonstrate that microsatallite markers are useful for population genetic studies of *A. flavus*. The extent to which hypotheses of random mating among VCGs of *MAT1-1* and *MAT1-2* is supported by these studies will be discussed.

429. Re-sequencing shows biased SNP distribution along 8 A. fumigatus chromosomes. Suman Pakala, Jessica Hostetler, Suchitra Pakala, Vinita Joardar, Paul Bowyer, David Denning, William C. Nierman, Natalie D. Fedorova J. Craig Venter Institute, Rockville MD, USA. Email: natalief@jcvi.org

Aspergillus fumigatus is the most common causative agent of invasive aspergillosis (IA), an invasive and deadly infection that affects mostly immunocompromised individuals. To enhance genomic resources available the A. fumigatus community, this study aims to provide (i) 2 additional reference genome sequences; (ii) RNA-Seq based genome annotation upgrades; and (iii) mutational profiling of drug resistant strains. Here we report the successful completion of the pilot phase of the project. To estimate the feasibility of the NGS approach, we first calculated the Illumina sequencing error rate at the 10X cut-off, which turns out to be extremely low (2.4E-06). Sequencing two more azole-susceptible isolates, A1163 and AF210, demonstrated high genetic variability in the A. fumigatus nuclear genome. Our preliminary analysis identified 57,000 and 77,000 SNPs in A1163 and AF210, respectively, relative to AF293. With a few exceptions, SNPs were strongly enriched in the arms of the chromosomes relative to the center. Further analysis identified over 50 highly variable genes (over 15 SNPs per 1 Kb) including the FluG-like protein and general amidase GmdB, which may be useful for MLST typing. Notably, the cyp51A gene, associated with azole resistance has 10 SNPs in AF210, some previously described as not related to drug resistance. Funding for the project has been provided by NIAIAD/NIH.

430. Intraspecific variation in effector genes of *Ustilago maydis*. Dominik Begerow, Ronny Kellner, Christian Hanschke. Ruhr-Universität Bochum, Geobotanik, Bochum, Germany. dominik.begerow@rub.de

Specific virulence factors regulate biotrophic key processes like penetration, resource tracking and specific disguise in the interaction of parasitic fungi and plants. To date, the mode of selection of fungal virulence factors and their impact on speciation are poorly understood. The corn smut *Ustilago maydis* lacks a classical gene-for-gene interaction with its host *Zea mays*, but genome data revealed a great number of probably secreted proteins which might be relevant for the pathogenic interaction. In order to find selection patterns in virulence cluster genes within *U. maydis*, we focused on gene clusters encoding secreted effectors that affect different stages of the biotrophic interaction. These included the hypervirulence-associated cluster 2A, partial sequences of cluster 19A associated with markedly reduced virulence and the fungal effector-encoding gene pep1. We estimated the intraspecific variability of 33 and 15 cluster genes encoding secreted and unsecreted proteins, respectively, as well as of internal transcribed spacer sequences, 5.8S rDNA and rpb1. In total, 51 loci from up to 16 strains have been sequenced either completely or partially. The genetic diversity of cluster genes reflected the reported population structure of *U. maydis*, although sequence divergence is low. However, potential virulence genes, encoding for secreted proteins, accumulated significantly more substitutions than genes of unsecreted proteins suggesting their differential selection during evolution.

431. Inherent and ecological constraints on the evolution of quantitative fitness traits in the rust fungus *Puccinia coronata*. Emily Bruns [1*], Martin Carson [2], and Georgiana May [1], [1]University of Minnesota, Saint Paul, MN [2] USDA-ARS Cereal Disease Lab, Saint Paul, MN [*]bruns094@umn.edu

In plant pathology, a large body of work has focused on the evolution of virulence, a qualitative trait allowing infection of otherwise resistant hosts, while relatively few studies have examined evolution of quantitative fitness traits, those affecting the reproductive success of the pathogen after infection has occurred. Yet, quantitative reproductive traits are a critical component of pathogen fitness, and a clear understanding of the relationship between virulence and quantitative reproduction traits will provide insight into potential constraints on the evolution of virulence. We investigated the effects of pathogen virulence and host genotype on fitness traits of *Puccinia coronata f.sp. avenae*, the causal agent of oat crown rust. We found that virulence level did not have a significant effect on pathogen fitness traits, but variation in host genotype significantly affected several pathogen fitness traits. Our results suggest that the evolution of virulence in *P. coronata*, a broadly distributed, sexually recombining plant pathogen is unlikely to be constrained by inherent fitness costs, but that ecological factors such as the host genotype and host diversity may play an important role.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

432. Fungi on *Abies grandis* wood. Navarro-Gonzalez, Monica and Kües, Ursula Geirg-August-Universität Göttingen, Germany

Abies grandis (Grand Fir) is a neophyte in Germany. Little is known about fungi infesting its wood. In this study we isolated fungi from dead wood from forests for molecular identification. Most species were either ascomycetes or related deuteromycetes but also a few basidiomycetes were detected, some of which are brown rots. Most of the isolates could only be identified to the genus level and some only to a family level, indicating a large range of unknown fungi occuring on dead wood. Generally, for most tree species dead wood has yet little been studied with molecular methods in terms of fungal occupants. Conservative estimates predict that 1.5 million different fungi exist worldwide, most of which are still undiscovered. The study showed that dead wood represents one of the biotops in which many missing fungal species can be detected. This work was supported by the BMBF (Germany).

433. Designing molecular markers for detection of the fish-pathogenic oomycete species *Saprolegnia parasitica*. Wai Lam (Webby) Leung, Paul de la Bastide and Will Hintz. Department of Biology, University of Victoria, PO Box 3020, Station CSC, Victoria, BC V8W 3N5 Canada (webby@uvic.ca)

Saprolegnia parasitica is an oomycete that causes saprolegniosis. Freshwater species are severely affected by this disease, leading to great losses in fish production for the aquaculture industry. In the past,*S. parasitica* infections had been controlled with malachite green; however, since this chemical was banned due to its potential carcinogenic effects, saprolegniosis has returned as a significant problem. Improved disease management requires the ability to accurately detect and quantify the causal agent. Historically, discrimination among species of the genus *Saprolegnia* was largely based on the morphological characteristics of sexual structures that are rarely observed in vitro; reliable species-level identification is thus very difficult to attain, based solely on these features. There is currently no DNA-based diagnostic test for the detection of this pathogen. We are developing species–specific molecular markers for *S. parasitica* and have initially focused our efforts on the Internal Transcribed Spacer (ITS) region. Both field- collected and reference isolates of *S. parasitica* are being studied to determine the suitability of this region. Unique regions of the ITS have been used to design species-specific primers for the rapid identification of *Saprolegnia* species. Additional gene loci are being considered for genetic marker development and may contribute to a multiplex marker system.

434. The discovery of a fungal hybrid front provides insights on general mechanisms of interspecific gene introgression. Matteo Garbelotto*, 2, and Paolo Gonthier, 1. 1 University of Turin, Italy 2 University of California, Berkeley, CA, USA

The paucity of known fungal species hybridizing has significantly hindered our understanding of the mechanisms driving gene introgression in these eukaryotic microbes. By investigating patterns of gene introgression between the invasive plant pathogen Heterobasidion irregulare introduced from North America and the native H. annosum in Italy, we describe a discrete and moving fungal front of hybridization and we quantify and qualify the observed introgression. STRUCTURE analysis of AFLPs conducted on 267 genotypes indicates hybridization and gene introgression in the invasion area can be documented in 8-42% of genotypes, depending on site. Data indicate that introgression is mostly occurring unilaterally from the native species to the invasive one, and is responsible for 5-45% of genomes in admixed genotypes. Sequence analysis of 11 loci for 30 genotypes determined that a significant majority of introgressed alleles ($P = 0.009$) was synonymous to alleles already found in the receiving species. However, the ratio of synonymous vs. non-synonymous introgressed alleles did not significantly differ ($P = 0.765$) from that occurring between the two species in the area of sympatry. These findings indicate that alleles are being introgressed across species boundaries independent of function, and provide the best evidence to date, and not just for fungi, in support of an introgression process that is driven by population expansion processes, rather than by selection.

435. The KP4 killer protein gene family. Daren W. Brown Bacterial Foodborne Pathogens and Mycology Research, USDA-ARS-NCAUR, 1815 N. University St., Peoria, Illinois 61604, USA, daren.brown@ars.usda.gov

Killer protein 4 (KP4) is a well studied viral toxin secreted by the maize smut fungus *Ustilago maydis* that kills sensitive *Ustilago* strains as well as inhibits *Fusarium* and plant root growth by inhibiting calcium uptake. Numerous small, cysteine rich proteins have been shown to play a critical role in fungal-plant-bacterial associations. The discovery of six KP4-like genes in *F. verticillioides* precipitated efforts to understand their function and evolutionary origin. Analysis of publicly available genomic sequence identified 31 additional KP4-like genes from a range of Ascomycota, a Basidiomycota, and the moss *Physcomitrella patens*. Sequence comparison and phylogenetic analysis indicate that the viral KP4 and the moss and fungal KP4-like genes evolved from a common ancestor providing evidence for lateral gene transfer between kingdoms. Six genes of the 37 total genes are predicted to encode a protein with two, non-identical KP4-like domains in tandem separated by 29 to 56 amino acids. The results indicate that two independent events led to the dual-domain KP4 genes present in different lineages of the Ascomycota. Understanding the nature and function of KP4-like proteins in mycotoxin-producing species like *Fusarium* may help to limit plant diseases and increase food safety by limiting toxin contamination and increase food production.

436. Ribosomal RNA gene evolution in arbuscular mycorrhizal fungi. Nicholas W. VanKuren[1], Henk C. den Bakker[1], Joseph B. Morton[2] & Teresa E. Pawlowska[1] [1]Department of Plant Pathology and Plant Microbe-Biology, Cornell University, Ithaca, New York 14853- 5904, USA; [2]Division of Plant and Soil Sciences, West Virginia University, Morgantown, West Virginia 26506, USA.

To understand how different evolutionary forces contribute to the patterns of molecular divergence in arbuscular mycorrhizal fungi (phylum Glomeromycota) and identify factors responsible for the evolutionary longevity of Glomeromycota, we studied evolution of rRNA genes in the *Glomus etunicatum* lineage. We examined the rRNA gene history, localization in interphase nuclei, and the effects of mutations on the rRNA structure and function. We also estimated effective population size in *G. etunicatum* and used this estimate as a platform to explain molecular diversity in this organism. We found that species in the *G. etunicatum* lineage exhibit three distinct patterns of rRNA gene evolution: (i) sharing of an interspecific ancestral polymorphism, (ii) coexistence of multiple polymorphic rRNA genes fixed in geographically differentiated populations, and (iii) concerted evolution within divergent rRNA gene clades. A modest effective population size in *G. etunicatum* suggests that mutation and random genetic drift play a dominant role relative to selection in this organism. In such modestly sized populations, neutral and slightly deleterious mutations are expected to fix rapidly in the absence of meiotic population-level rRNA gene homogenization, which results in a pattern of intraspecific and intraindividual rRNA gene divergence typical in Glomeromycota. Physical separation between divergent rRNA gene arrays in interphase nuclei contributes to imperfect rRNA gene homogenization by concerted evolution. Accumulation of compensatory mutations evident in the rRNA genes damaged by deleterious mutations suggest that compensatory evolution may be an important mechanism that protects Glomeromycota from extinction.

437. Population genomics and local adaptation in *Neurospora crassa* isolates from the Caribbean Basin. Christopher E. Ellison[1], Charles Hall[1], David Kowbel[1], Juliet W. Welch[1], Rachel B. Brem[2], N. Louise Glass[1], John W. Taylor[1] Departments of [1]Plant & Microbial Biology and [2]Molecular and Cell Biology, University of California, Berkeley, CA 94720-3102, USA. cellison@berkeley.edu

The elucidation of the genetic basis of adaptation is a highly sought after, yet rarely achieved goal. Thus far, most instances where adaptive alleles have been discovered involved identifying candidate genes based on their having a function related to an obvious phenotype such as pigmentation. This "forward-ecology" approach is difficult for most fungi because they lack obvious phenotypes. We have used a "reverse-ecology" approach to identify candidate genes involved in local adaptation to cold temperature in two recently diverged populations of *Neurospora crassa* by performing high-resolution genome scans between populations to identify genomic "islands" of extreme divergence. We find two such islands containing genes whose functions, pattern of nucleotide polymorphism, and null phenotype are consistent with local adaptation.

438. Withdrawn

439. Evolution of pathogenicity in *Rhizoctonia* fungi. M. Rodriguez-Carres[1,2], A.K. Nakatani[3], R. Vilgalys[2], T. Boekhout[4], J.A. Stalpers[4], E.E. Kuramae[4], N.L. Souza[3], S. Mandava[2], and M.A. Cubeta[1]. [1]North Carolina State University, Department of Plant Pathology, Raleigh, NC. [2]Duke University, Department of Biology, Durham, NC. [3]UNESP, Faculdade de Ciencias Agronomicas, SP, Brazil. [4]CBS Fungal Biodiversity Centre, Utrecht, The Netherlands.

The genus *Rhizoctonia* is an economically important group of soil fungi that includes host specialized and generalist pathogens of plants and mycorrhizal symbionts of liverworts, pine, mosses and orchids. Identification and delineation of *Rhizoctonia* species has been challenging due to the limited number of informative morphological characters associated with their sexual and asexual stages. *Rhizoctonia* fungi are typically classified into anastomosis groups (AG) based on hyphal vegetative compatibility assays with tester strains. Previously molecular studies suggested that AGs likely represent independent evolutionary and monophyletic groups that belong to the Cantharelloid clade. The Cantharelloid clade includes morphologically diverse species that are saprobic (*Botryobasidium* and *Sistotrema*), lichenicolous (*Burgoa*), orchid endomycorrhizal (*Tulasnella*), and ectomycorrhizal (*Cantharellus*). In this study, a multi-locus sequencing approach using five loci (ITS, LSU, *RPB*2, *EF*1alpha, and *ATP*6) was conducted to construct a phylogenetic framework to evaluate morphological and trophic transitions within the *Rhizoctonia* species complex and to examine relationships among isolates belonging to different AGs that differ in predominant trophic behavior (plant pathogenic, saprobic, and orchid mycorrhizae). Current results suggest that 1) plant pathogenic *Rhizoctonia* fungi are represented by several rapidly diverging groups and 2) the family *Ceratobasidiaceae*, which includes the majority of *Rhizoctonia* anamorphs, is a sister group to the Cantharelloid clade. Based on these finding we propose that the pathogenic *Rhizoctonia* are derived from a plant symbiotic ancestor(s) that also gave rise to the Cantharelloid clade.

440. Sexual recombination and the possibility of cryptic heterokaryosis in *Aspergillus flavus*. Rodrigo A. Olarte[1], Bruce W. Horn[2], James T. Monacell[3], Rakhi Singh[1], Eric A. Stone[3,4], Ignazio Carbone[1]. [1]Plant Pathology, NCSU, Raleigh, NC 27695 [2]NPRL, USDA-ARS, Dawson, GA 39842 [3]BRC, NCSU, Raleigh, NC 27695 [4]Genetics, NCSU, Raleigh, NC 27695

Aspergillus flavus infects both plants and animals and is of toxicological importance due to its production of aflatoxins (AFs). Recent efforts to reduce AF concentrations have focused on the use of the biocontrols AF36 and Afla- Guard®, both of which contain nonaflatoxigenic *A. flavus* strains as an active ingredient. Biocontrols are applied to fields, where they competitively exclude native aflatoxigenic strains. Although biocontrol is effective in reducing AF contamination in crops, the extent to which these strains recombine with native strains and the overall effect on fungal populations are unknown. Here we show that the recombination breakpoints in the F1 correlate with the breakpoints inferred from population genetic studies of natural isolates. Furthermore, we demonstrate that a crossover event within the AF cluster can repair a nonsense mutation, resulting in a regained aflatoxin-producing phenotype. Finally, we observed non-Mendelian inheritance of extra-genomic AF cluster alleles in crosses with partial AF cluster parents, suggesting a possible role of cryptic heterokaryosis, in addition to sexual recombination, in modulating AF production. Collectively, these processes may contribute to increased effective population sizes and drive genetic and functional hyperdiversity in *A. flavus*.

441. Role of Viral and Plasmidic Genes in the genetic diversity of the Glomeromycota. Laurence Daubois, Eric Bapteste, Philippe Lopez, Mohamed Hijri and Francois-Joseph Lapointe. Plant Biology Research Institute, University of Montreal, Montreal, Canada and UMR7138, University of Paris VI, Paris, France. laurence.daubois@umontreal.ca

Arbuscular mycorrhizal fungi (AMF) are widespread plant symbionts helping plants to acquire nutrients such as phosphorus and nitrate, and increasing their resistance to soil pathogens. Therefore, AMF play an important role in the plant's growth. These fungi contain several thousands of genetically different nuclei, freely circulating throughout their coenocytic hyphae, and form spores that are always multinucleated. The absence of known sexuality in these organisms questions what mechanisms could be implicated in such a high intra-isolate genetic diversity. In this study, we report the presence of mobile elements genes (viruses and plasmids) in two different AMF isolates : *Gigaspora rosea* and *Glomus diaphanum*. Contigs of those two isolates were produced from pyrosequencing data, and genes homologies with viruses and plasmids (from NCBI) were detected using network based analyses. Some of the viral/plasmidic connections with AMF were consistent with their lifestyle, suggesting the transfer of genetic material between mobile elements and AMF as a possible source of genetic diversity. We also observed that the isolate with a larger number of nuclei in its spores contained a larger number of genes homologous with mobile elements. A weaker genetic drift in such AMF might explain the accumulation these genes in their nuclei, and maybe a part of the AMF genetic diversity.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

442. Hybridization between *Aspergillus flavus* and *Aspergillus parasiticus*. Carolyn J. Worthington[1], Bruce W. Horn[2], Geromy G. Moore[1], James T. Monacell[3], Rakhi Singh[1], Eric A. Stone[3,4], Ignazio Carbone[1]. [1]Plant Pathology, NCSU, Raleigh, NC 27695 [2]NPRL, USDA-ARS, Dawson, GA 39842 [3]BRC, NCSU, Raleigh, NC 27695 [4]Genetics, NCSU, Raleigh, NC 27695

To date the sexual stages or teleomorphs have been described for three aflatoxigenic species in *Aspergillus* section *Flavi: Petromyces flavus, P. parasiticus* and *P. nomius*. In this study we examined the possibility of interspecific matings between *A. flavus* and *A. parasiticus*. These species can be distinguished morphologically and genetically, as well as functionally via the biosynthesis of specific aflatoxins and an unrelated mycotoxin, cyclopiazonic acid (CPA). *A. flavus* produces both B aflatoxins and CPA, B aflatoxins or CPA alone, or neither mycotoxin; *A. parasiticus* produces B and G aflatoxins or O-methylsterigmatocystin but not CPA. Only four out of forty-five attempted interspecific crosses between compatible mating types of *A. flavus* and *A. parasiticus* were fertile. Single ascospore strains from each cross were isolated and shown to be hybrids using array comparative genome hybridization and multilocus sequence typing. Higher mean concentrations of B_1, B_2, G_1 and G_2 aflatoxins, and CPA in the F1 progeny compared to midpoint parent toxin levels indicate high heritability of these toxins and possibly hybrid heterosis.

443. Global genetic structure of the fungal grapevine pathogen *Eutypa lata*. Renaud Travadon[1], Kendra Baumgartner[2], Philippe Rolshausen[3], Walter Douglas Gubler[1], Mark Sosnowski[4], Pascal Lecomte[5], Francois Halleen[6] and Jean-Pierre Péros[7]. [1]University of California, Davis, USA, rtravadon@ucdavis.edu. [2]USDA-ARS, Dept. of Plant Pathology, Univ. of California, Davis, USA. [3]University of California, Riverside, USA. [4]South Australian Research and Development Institute, Adelaide, Australia. [5]INRA, UMR Santé Végétale,Villenave d'Ornon, France. [6]ARC Infruitec-Nietvoorbij, Stellenbosch, South Africa. [7] INRA, UMR DIAPC, Montpellier, France.

The ascomycete fungus *Eutypa lata* is a trunk pathogen of cultivated grapevine (*Vitis vinifera*) in all major grape-growing regions of the world. Throughout its geographic range, it is considered a generalist pathogen that can complete its life cycle on a broad range of hosts. To decipher the cosmopolitan distribution of this fungus, we investigated the population genetic structure of 19 geographic samples from four continental regions (Australia, California, South Africa and Europe), based on analyses of 287 isolates genotyped with nine microsatellite markers. High levels of genotypic diversity and absence of multilocus linkage disequilibrium among loci supported the preponderance of sexual reproduction in all regions examined. Nonetheless, the identification of identical multilocus haplotypes with identical vegetative compatibility groups, in vineyards in California and South Africa, suggests that dispersal of asexual spores of the fungus could be a rare means of disease spread. The greatest levels of allelic richness and gene diversity were found in Europe, among geographic samples from coastal areas surrounding the Mediterranean Sea, whereas the lowest genetic diversity was found in South Africa. Samples from California, Australia and South Africa, which had lower genetic diversity than those of Europe, were also characterized by demographic disequilibrium and, thus, may represent founding populations of the pathogen. Low levels of genetic differentiation among all samples suggest that gene flow prevents differentiation at continental scales. These findings suggest that global, human-mediated spread of the fungus may have resulted in its currently global distribution.

444. Horizontal gene transfer in the evolutionary history of ammonium transporters/ammonia permeases in fungi. McDonald, Tami[1], Fred Dietrich[2], and Francois Lutzoni[1]. [1]Biology Department, Duke University, USA. [2] Department of Molecular Genetics and Microbiology, Duke University, USA.

The proteins of the ammonium transporter/methylammonium permease/Rhesus factor family (AMT/MEP/Rh family) are responsible for the movement of ammonia or ammonium ions across the cell membrane. We use phylogenetic analysis to infer the evolutionary history of this family of proteins across 191 genomes representing all main lineages of life. We show that the AMT/MEP/RH family illustrates contrasting modes of gene transmission: the AMT family is characterized by vertical gene transmission (i.e. the gene phylogeny matches the organismal phylogeny), whereas the MEP family as defined here is characterized by several ancient independent horizontal gene transfers, including two into the fungi. Soon after the origin of the fungi, the transfer of a MEP family transporter from bacteria replaced the ancestral AMT family transporter. An additional MEP family transporter was transferred from hyperthermophilic chemoautolithotrophic prokaryotes to the filamentous ascomycetes associated with the rapid radiation of the leotiomyceta, which have subsequently lost this extra transporter unless lichenized. We detected this transporter in lichenized Lecanoromycetes, Dothidiomycetes and Eurotiomycetes, including the model lichen *Cladonia grayi*. The gene clades as depicted in this phylogenetic study appear to correspond to functionally different groups, with ammonium transporters and ammonia permeases forming two distinct monophyletic groups.

445. Mating-Type and Sexual Potential of the Black Aspergilli. Heather Darbyshir[1], Rob Samson[2], Janos Varga[2,3], Scott Baker[4], Sammy Ladhar[1], Peter van de Vondervoort[5] & Paul Dyer[1] [1]School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD UK. [2]CBS Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. [3]Department of Microbiology, Faculty of Science and informatics, University of Szeged, H-6726 Szeged, Közép fasor 52, Hungary. [4]Pacific Northwest National Laboratory, Fungal Biotechnology Team, Richland, WA 99352, U.S.A. [5]DSM Food Specialties, PO Box 1, 2600 MA Delft, The Netherlands.

The black aspergilli are members of the genus *Aspergillus* that are typically characterized by the production of black conidia. The group includes *Aspergillus niger*, which is of particular industrial importance because of its safe use and ability to produce a wide range of enzymes and organic acids. All black aspergilli are currently known to reproduce only by asexual means. However, sexual cycles have recently been discovered in other aspergilli which were previously considered asexual. The discovery of a sexual cycle in *A. niger* and related species would provide a valuable tool for strain improvement and provide insights into their population biology and evolution. Preliminary screening of the black aspergilli has revealed the presence of mating-type genes, which are key transcriptional regulators of sexual reproduction. Using mating-type sequences available from genome databases, together with experimentally derived idiomorph data, a diagnostic multiplex PCR test was designed to allow determination of mating-type of *A. niger* and closely related black aspergilli. Mating-type screening has indicated a *MAT1-1* bias in *A. niger* and a *MAT1-2* bias in *A. carbonarius*. The detection of both mating-types amongst certain species of the group indicates that the black aspergilli includes species that are, or have once been, heterothallic (obligate outbreeding) sexual species. Certain black aspergilli have also been shown to form sclerotia, structures associated with sexual reproduction in the teleomorph genus *Petromyces*, again consistent with past sexuality. Work is now ongoing to determine best conditions to induce sclerotial development as a prerequisite for sex.

Pathogenic and Symbiotic Interactions

446. Disruption of *Colletotrichum acutatum nox2* reduces fungal pathogenicity on strawberry. Tomer Gershon[1,2,*], Oded Yarden[1], and Stanley Freeman[2,]. [1]Dept. of Plant Pathology and Microbiology, The R.H. Smith Faculty of Agriculture, Food & Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel. [2]Dept. of Plant Pathology and Weed Research, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel. tomer@volcani.agri.gov.il

The fungus *Colletotrichum acutatum* J.H. Simmonds (*C.a*) is the major causal agent of strawberry anthracnose. Previously it was established that the early stage of *C.a*-strawberry interaction requires reactive oxygen species (ROS) production. Since ROS, produced by NADPH oxidase (NOX) is a major factor that may be involved in pathogenicity, the aim of this study was to assess the effect of two different NOX genes (*nox1*, *nox2*) on disease and development of the pathogen by reverse genetics. Full genomic sequences of NOX genes were generated and used as a basis for relative quantification of *nox* m-RNA expression and preparation of disruption cassettes for gene replacement experiments. Real-time-PCR experiments revealed a significantly higher expression of the *nox2* gene relative to *nox1*, in all fungal tissues but mainly in conidia. Mutant strains Ca1.5 and Ca2.3 were generated for *nox1* and *nox2*, respectively. Ca1.5 grew more rapidly than the wildtype isolate whereas less conidia were produced by this mutant. No differences were evident between strain Ca2.3 and the wildtype isolate regarding growth rate and conidia production. Surprisingly, strain Ca2.3 which appeared unaffected in development, was nonpathogenic on detached strawberry leaves and caused reduced symptom development on strawberry daughter plants, whereas strain Ca1.5 remained fully pathogenic. Since no *noxc* gene homologs were identified in *C.a*, a double *nox1:: nox2* mutant will be generated and gel NOX activity assays and pathogenicity tests will be conducted on strawberry seedling to verify no additional NOX activity.

447. Oxidative Stress Sensing and Signalling in the *Epichloë festucae - Lolium perenne* Symbiosis. Gemma Cartwright[1], Yvonne Becker[1], Barry Scott[1]. [1]Massey University, Palmerston North, New Zealand.

Reactive oxygen species (ROS) are both toxic by-products of cellular metabolism and important signalling molecules. The mutualistic association formed between *Epichloë festucae* and *Lolium perenne* is dependent on the generation of ROS by the fungal NADPH oxidase NoxA (Tanaka *et al*., 2006). In *Saccharomyces cerevisiae* oxidative stress is sensed via a redox relay system comprising the thiol peroxidase GPX3 and transcription factor YAP1 (Delaunay *et al*., 2002). Here, the roles of *E. festucae* YapA and GpxC, homologues of *S. cerevisiae* YAP1 and GPX3, in oxidative stress signalling are investigated. Full-length cDNAs encoding *E. festucae* YapA and GpxC were cloned into the pYES2 vector and transformed into *S. cerevisiae* Δ*YAP1* and Δ*GPX3* strains to test whether they would complement the H_2O_2-sensitivity of the *S. cerevisiae* mutants. *E. festucae* YapA and GpxC were able to substitute for the *S. cerevisiae* homologues, YAP1 and GPX3. To analyse the functions of these proteins in *E. festucae*, deletion constructs were prepared using *hph* and *nptII* as selectable markers for replacement of *yapA* and *gpxC*, respectively. Growth of the Δ*yapA* and Δ*gpxC* strains was analysed in a range of stress-inducing conditions. The *yapA* deletion mutants showed increased sensitivity to menadione and diamide. *L. perenne* plants infected with Δ*yapA* mutants show increased tillering, however this phenotype is highly variable. A YapA-GFP fusion protein relocalises from the cytoplasm to the nucleus following treatment with menadione and H_2O_2 in both wild-type and Δ*gpxC* strains. Future work will include the use of clonal plant lines to overcome the plant phenotype variability associated with infecting highly heterozygous *L. perenne* seedlings with the *yapA* mutant.

448. The Trehalose Pathway contributes to key virulence attribute production in *Aspergillus fumigatus.* * Srisombat Puttikamonkul1, Sven D. Willger1, Nora Grahl1, John R. Perfect2, Navid Movahed3, Brian Bothner3, and Robert A. Cramer Jr.1* 1Department of Veterinary Molecular Biology, Montana State University, Bozeman, MT 59718, 2Department of Medicine, Duke University Medical Center, Durham, NC 27713, 3Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59718 rcramer@msu.montana.edu

Recently, our studies on OrlA (Trehalose 6 Phosphate (T6P) Phosphatase) in *A. fumigatus* suggest that increases in T6P levels lead to decreased activity of hexokinase, abolished asexual reproduction, cell wall defects, and avirulence in murine models of IPA. However, these phenotypes are not due to loss of trehalose itself since production persists in the absence of OrlA through an unknown mechanism. Moreover, complete loss of T6P and trehalose production only occurs when 2 genes, TpsA and TpsB, encoding trehalose 6 phosphate synthases, are both deleted. Intriguingly, loss of TpsA but not TpsB in strain CEA10 results in cell wall defects that affect fungal pathogenesis further supporting a link between trehalose metabolism and cell wall dynamics in *A. fumigatus*. Consequently, we hypothesize that the trehalose biosynthesis pathway and its intermediates play key roles in regulating fungal cell wall biosynthesis by affecting central carbon metabolism of fungi. These affects consequently have significant ramifications for the ability of *A. fumigatus* to cause disease. Further genome sequence analysis of the *A. fumigatus* genome reveals additional uncharacterized genes predicted to encode proteins likely involved in trehalose biosynthesis and metabolism. Amino acid alignments of these genes suggests that they may play important undefined roles in regulating trehalose production underscoring the complexity and unknown mechanisms of action of this pathway in *A. fumigatus*. Therefore, our results strongly suggest that trehalose biosynthesis and catabolism are important components of *Aspergillus* biology that directly affect fungal pathogenesis by as yet undefined mechanisms.

449. Analysis and Sorting of A. niger pellets based on size and fluorescence. Weon Bae, Rico Bongaarts, and Rock Pulak. Union Biometrica, Holliston, MA

Aspergillus niger is one of the most common species of the genus *Aspergillus*. Some species of *Aspergillus* are pathogenic to both human and animals, whereas other species are very important in the commercial production of natural products, which can be used to develop biosensors and medications. Various strains of *A. niger* have been used for mass production of citric acid, gluconic acid, and many enzymes. For these purposes, *A. niger* is preferably cultured in liquid medium in a bioreactor for the maximal productivity. When they are grown in liquid culture, they form sub-millimeter micro-colonies with significant heterogeneity in size. It was also reported that *A. niger* heterogeneously expresses many genes encoding secreted proteins, which degrades the organic matter to use as nutrients (de Bekker et al). The COPAS instruments are special flow cytometer to analyze and sort large objects up to 1500 microns in diameter. We report here that the COPAS PLUS platform, which is able to handle objects between 30 and 700 microns, was successfully used to analyze and sort individual *A. niger* pellets by size (TOF), optical density (EXT), green fluorescence (GFP), and red fluorescence (dTomato) signals. This will enable researchers to study the genetic basis of heterogeneity in *A. niger* grown in liquid culture. The COPAS technology could also be used to study why pellets grow in different size, what the mechanism is behind it, and how it effects production.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

450. The MEEK-related CpBck1 in cell wall integrity of the chestnut blight fungus *Cryphonectria parasitica*. Dae-Hyuk Kim, Joong-Gi Lee, Jung-Mi Kim, Seung-Moon Park. Institute of Molecular Biology and Genetics, Center for Fungal pathogenesis, Chonbuk National University, Jeonju, Korea.

*Cpbck*1, encoding a mitogen-activated protein (MAP) kinase kinase kinase from the chestnut blight fungus *Cryphonectria parasitica*, is an ortholog of Bck1 from *Saccharomyces cerevisiae*. To characterize biological function of the *Cpbck*1 gene in the *C. parasitica*, *Cpbck*1-null mutant was constructed using the replacement vector designed to favor double-crossover integration events. Colony morphology of the *Cpbck*1-null mutants differed dramatically from the wild type that mutants showed the invasive growth pattern characterized by slower growth rate, absence of distinctive aerial hyphae resulting in almost absence of conidia-bearing structure and conidia, sparse mycelial growth on the surface of agar plate with abnormal pigmentation, and irregular mycelial mat within the restricted area. Feeding hyphae growing under the plate showed less branched and relatively slower growth pattern. Interestingly, the *Cpbck*1-null mutant produced sectors appeared as thick, rubbery patches of matted growth without pigmentation and sporulation. In addition, these characteristics of sector were maintained when they were transferred into a new plate. Intracellular structure observed by electron microscope revealed both invasive growth-type and sectored-type showed the occurrence of hypertrophy of cell wall, multiple nuclei within swollen cells and intrahyphal hyphae. The hyphae of the *Cpbck*1-null mutant showing the invasive growth showed both hyphal tip swelling as well as atypical branches emerging below the swellings. However, the hyphae of the sector showed more compact mycelial distribution with a lot of aggregation of individual hypha. It will be of interest to determine what decide the transition of the mycelia growth pattern from the invasive and very-sick hyphal growth type to compact-mat type.

451. The tetraspanin *FgPls1* is involved in fitness and pathogenicity of *Fusarium graminearum*. Cornelia L Staerkel Wilhelm Schäfer. Molecular Phytopathology and Genetics, University Hamburg,Germany

Tetraspanins are a family of small membrane proteins specific to animals and fungi. These proteins with characteristic secondary structures are involved in a broad range of biological processes. They behave as “molecular facilitators” interacting with other membrane proteins such as integrins, adhesion proteins, metalloproteases and proteins with Ig domains in animals. In fungi, three different families of tetraspanins were characterized. Pls1 is present in ascomycota and basidiomycota while Tsp2 is unique to basidiomycota, and Tsp3 is unique to ascomycota. Pls1 null mutants from plant pathogenic fungi such as *Magnaporthe grisea*, *Botrytis cinerea*, and *Colletotrichum lindemuthianum* are non pathogenic on plants being defective in appressorium mediated penetration. In this study, we identified FgPLS1, the functional orthologue of MgPLS1 in the wheat scab fungus *F. graminearum*. Null mutants obtained by targeted gene replacement displayed defects in pathogenicity and additional phenotypes (altered mycelium growth, highly reduced production of macroconidia) not observed in other fungal PLS1 mutants. These results demonstrate that this gene is important for vegetative growth, sporulation, and pathogenicity in *F. graminearum*. Therefore, although Pls1 tetraspanins control cellular functions involved in infection conserved among fungal plant pathogens, they have been recruited to control cellular functions involved in growth and sporulation specifically in *F. graminearum*.

452. Epigenetic gene silencing of *Phytophthora sojae* effectors. Dinah Qutob, Irina Mutiu, Kuflom Kuflu, Patrick Chapman, and Mark Gijzen. Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON, Canada, N5V 4T3

A pivotal development in understanding pathogen evasion of host recognition has been the identification of several avirulence (*Avr*) genes encoding RxLR effectors in the soybean pathogen, *Phytophthora sojae*. Analysis of *P. sojae Avr* genes has revealed several mechanisms through which the pathogen has evolved the ability to achieve a gain of virulence in plants carrying the corresponding resistance genes. Gain of virulence mutations for *P. sojae Avr* genes include amino acid changes, gene deletions, promoter and 5' UTR mutations, and transcriptional variation. The genes *Avr1a* and *Avr3a* display transcriptional polymorphisms between *P. sojae* strains, despite that the corresponding gene sequences are, in certain cases, identical. We followed the segregation patterns of these gene silenced loci by performing crosses between *P. sojae* strains and tracing the pattern of effector gene expression and virulence in the F_1 and F_2 progeny. Depending on the cross, gene silenced alleles showed normal Mendelian recessive segregation or unusual paramutagenic inheritance patterns. We conclude that naturally occurring paramutation in *P. sojae* represents an important mechanism for generating variation in effector gene expression.

453. Preventing Fusarium Head Blight of Wheat and Cob Rot of Maize by Inhibition of Fungal Deoxyhypusine Synthase. Mayada Woriedh, Ilona Hauber, Ana Lilia Martinez-Rocha, Christian Voigt, Frank J. Maier, Marcus Schröder, Chris Meier, Joachim Hauber, Wilhelm Schäfer Molecular Phytopathology, Biocenter Flottbek, University Hamburg schaefer@botanik.uni-hamburg.de

Upon posttranslational activation, the eukaryotic initiation factor-5A (eIF-5A) transports a subset of mRNAs out of the nucleus to the ribosomes for translation. Activation of the protein is an evolutionary highly conserved process which is unique to eIF-5A: the conversion of a lysine to a hypusine. Instrumental for the synthesis of hypusine is the first of two enzymatic reactions mediated by deoxyhypusine synthase (DHS). We show that DHS of wheat and the pathogenic fungus Fusarium graminearum, which causes one of the most destructive crop diseases worldwide, are transcriptionally upregulated during their pathogenic interaction. Although DHS of wheat, fungus, and human can be equally inhibited by the inhibitor CNI-1493 in vitro, application during infection of wheat and maize flowers results in strong inhibition of the pathogen without interference with kernel development. Our studies provide a novel strategy to selectively inhibit fungal growth, without affecting plant growth. We identified fungal DHS as a target for the development of new inhibitors, for which CNI-1493 may serve as a lead substance.

454. Cellular pathways activated in the necrotrophic fungus Alternaria brassicicola in response to camalexin exposure. Thomas Guillemette, Aymeric Joubert, Claire Campion, Nelly Bataillé-Simoneau, Beatrice Iacomi-Vasilescu, Pascal Poupard, Philippe Simoneau. IFR QUASAV, UMR PaVé 77, 2 Bd Lavoisier, F 49045 Angers, France

Camalexin, the characteristic phytoalexin of Arabidopsis thaliana, inhibits growth of the fungal necrotroph Alternaria brassicicola. This plant metabolite probably exerts its antifungal toxicity by causing cell membrane damage. Here we observed that activation of a cellular response to this damage requires the unfolded protein response (UPR) and two MAPK signalling pathways, the cell wall integrity (CWI) and the high osmolarity glycerol (HOG). Camalexin was found to activate both AbHog1 and AbSlt2 MAP kinases in a precocious manner, and activation of the latter was abrogated in an AbHog1 deficient strain. Mutant strains lacking functional MAP kinases or AbHacA, the major UPR transcription regulator, showed in vitro hypersensitivity to camalexin and brassinin, a structurally related phytoalexin produced by several cultivated Brassica species. Enhanced susceptibility to the membrane permeabilization activity of camalexin was also observed for deficient mutants. These results suggest that the three signalling pathways have a pivotal role in regulating a cellular compensatory response to preserve cell integrity during exposure to camalexin. Replacement mutants exhibited a loss or an attenuation of the virulence on host plants that may partially result from their inability to cope with defence metabolites such as indolic phytoalexins. This constitutes the first evidence that a phytoalexin activates fungal MAP kinases and UPR, and that outputs of activated pathways contribute to protecting the fungus against antimicrobial plant metabolites. A functional model of fungal signalling pathways regulated by camalexin is proposed and leads to consider new promising strategies for disease control.

455. A T-DNA induced deletion identifies a host colonization locus in the grass symbiont *Epichloë festucae.* Milena Mitic, Murray Cox and Barry Scott Massey University, New Zealand. d.b.scott@massey.ac.nz

Epichloë festucae is a filamentous fungus that forms a mutualistic symbiotic association with Festuca and Lolium grasses. To identify genes required for host colonization and establishment we have used a forward genetics approach using *Agrobacterium tumefaciens* T-DNA induced mutagenesis. Among 200 hygromycin resistant transformants screened, one mutant was identified (Ag212) that had reduced radial growth in culture and was unable to colonize perennial ryegrass. Southern blot analysis showed that Ag212 has a single T-DNA insertion. Plasmid rescue was used to isolate sequences flanking the T-DNA left and right border sequences of the T-DNA insertion. Mapping of these junctions to the *E. festucae* 2368 genome sequence demonstrated the junctions were on different contigs indicating the presence of a genomic deletion. To determine the size of the deletion and the genes affected in this mutant, we used comparative synteny analysis with the genome of *Fusarium graminearum* in combination with Southern blotting and Illumina sequencing of the cosmid. This analysis revealed that Ag212 has a 25-kb deletion that includes 10 genes. Complementation tests using cosmids that span the deletion identified candidate genes for both the culture and in planta phenotype. Light and confocal microscopy analysis of seedlings infected with Ag212 containing a GFP expressing plasmid demonstrated that Ag212 is defective in cell wall attachment at the infection site and is completely deficient in host plant colonization.

456. Signaling genes in *Magnaporthe griesa.* Leethaniel Brumfield III, Doug Brown, Ralph A Dean. NC State University.

It is estimated that the world needs to produce 40% more rice by 2030 to feed its more than five billion rice consumers. Fungal disease, particularly that caused by the rice blast fungus Magnaporthe oryzae, is a major factor limiting rice production. A key to controlling rice blast disease is a better understanding of M. oryzae's pathogenic mechanisms; an important part of which resides in the ability of cellular signaling molecules (kinases) to phosphorylate a core set of transcription factors (TF) in a direct and controlled manner. Therefore, the more we know about the downstream targets of kinases, their associated pathways, and TF-regulated genes, the more effective controlling pathogenicity efforts will be. Previous pathway and network structure research in S. cerevisiae and H. sapiens may be utilized to better understand cellular signaling in M. oryzae when investigating similar proteins. Large scale protein phosphorylation microarrays can be used to accurately identify functional TF targets of homologous kinases across these species. Potentially phosphorylated binding motifs were identified in these TFs using the Pratt algorithm that detects sequence patterns. These TF phosphorylation motifs were used to examine the shared functionality between homologous kinases. Such motifs may also provide potential chemical targets and aid in developing disease control strategies. Our findings showed that in all three species there were slightly more kinases that fell into the MAPK kinases family, and within M. oryzae enriched MAPK TFs reached 75.64% and 77.54% and 90.78% in H. sapiens and S. cerevisiae respectively.

457. A proteomics approach to dissect SnToxA effector mode-of-action in wheat Delphine Vincent[1], Ulrike Mathesius[1], Richard Lipscombe[2], Richard P. Oliver[3], Peter S. Solomon[1] [1]Research School of Biology, College of Medicine, Biology and Environment, The Australian National University, ACT Australia; [2]Proteomics International, Perth, WA, Australia; [3]Curtin University of Technology, Perth, WA, Australia delphine.vincent@anu.edu.au

Stagonospora nodorum is a necrotrophic fungal pathogen of wheat causing devastating foliar damage resulting in significant yield losses globally. *S. nodorum* operates in inverse gene-for-gene manner through the interaction of a secreted effector and a dominant host susceptibility protein resulting in disease development. The effector protein SnToxA and its corresponding host gene Tsn1 follow such a system. This study aims at deciphering the molecular responses triggered by SnToxA in the wheat (*Triticum aestivum*) susceptible cultivar BG261 over a 0-48hrs time course using a gel-based proteomics strategy. Wheat leaves were infiltrated with SnToxA and sampled at 0, 0.5, 4, 12, 24, and 48 hrs post-infiltration (hpi). Both acidic and basic proteins were studied and they generally display an up- regulation at 12 hpi followed by a down-regulation at 48 hpi. Differentially-expressed proteins are predominantly involved in energy and protein metabolisms, with many of the proteins identified localised in the chloroplast. The identities of these proteins and their possible roles will be discussed.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

458. Genetics of horizontal chromosome transfer in the plant pathogenic fungus *Fusarium oxypsorum*. Ido Vlaardingerbroek, H. Charlotte van der Does and Martijn Rep. Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, P.O. Box 94215, 1090 GE Amsterdam, The Netherlands.

The *Fusarium oxysporum* species complex is comprised of a many host specific asexual lineages. Interestingly, different lineages that infect a single host, reside in different phylogenetic clades. This distribution of host specificity has been attributed to acquired pathogenicity. Lineages sharing a host share chromosomes and genomic regions carrying effector genes. Transfer of these regions could explain the distribution of host specific lineages within the species complex. Horizontal transfer of complete chromosomes has been demonstrated under laboratory conditions, and resulted in gain of pathogenicity by the non-pathogenic recipient. Using a genetics approach we aim to uncover the underlying cellular processes, which have so far remained elusive. We are currently creating knockout strains in genes with key functions in processes thought be required for horizontal chromosome transfer. Furthermore, we will investigate what properties make a chromosome amenable for transfer. A screening method based on random insertion of a marker in the donor strain, will allows us to determine which chromosomes can be transferred. Analysis of these chromosomes should give us better insight into their unique properties. Combining knowledge of both the properties required for chromosome transfer and the cellular processes involved will help to gain a better understanding of this process and its importance in the evolution of the *F. oxysporum* species complex.

459. Evolution of lineage-specific chromosomes in the *Fusarium oxysporum* species complex Sarah M. Schmidt[1], Li-Jun Ma[2,3], H. Corby Kistler[4] and Martijn Rep[1] [1] Plant Pathology, SILS, University of Amsterdam, 1090 GE Amsterdam, The Netherlands [2] Broad Institute, Cambridge, MA, [3] University of Massachusetts Amherst, MA, USA, [4] Plant Pathology, ANRS, 6030 St Paul Campus, MN, USA.

Fusarium oxysporum is a soilborne fungus that causes wilt disease in many plant species by colonizing the host xylem vessels. The *F. oxysporum* species complex is a collection of apparently asexual non-pathogenic and pathogenic clonal lineages. Many lineages harbor unique genomic sequences residing mostly on extra chromosomes. In the tomato wilt strain *F. oxysporum* f. sp. *lycopersici* (*Fol*) the phylogenetic history of these lineage- specific (LS) chromosomes differs from the core genome. To investigate the origin of the LS chromosomes and their relation to host specificity within the *F. oxysporum* species complex we are currently analyzing genomic sequences of strains with host specificities towards Arabidopsis, cotton, human, pea, banana, cabbage, radish and melon. This genomics approach is complemented by a proteomics approach. In the interaction between *Fol* and tomato, the fungal effectors that are secreted in the xylem sap are crucial determinants of virulence and are encoded on LS chromosomes. We are presently investigating *Fo* f. sp. *melonis* effectors that are secreted during melon infection, as a first step towards assigning a virulence function to LS genes in this pathogenic strain.

460. A novel type I-interferon response modulates Candida virulence in mice and requires phagosomal TLR7 signaling in innate immune cells. Christelle Bourgeois, Olivia Majer, Ingrid E. Frohner, Iwona Lesiak-Markowicz, Walter Glaser, Silvia Stockinger, Thomas Decker, Shizuo Akira, Mathias Müller & Karl Kuchler

Human fungal pathogens such as Candida species (spp) can cause both superficial infections, as well as systemic candidiasis of high mortality in immunocompromised patients. The most frequent species are the dimorphic *Candida albicans* (Ca) and yeast-like *Candida glabrata* (Cg). Innate immune cells such as dendritic cells and macrophages establish the first line of defence against microbial pathogens and determine the outcome of infection. These cells release IFN-β, a type-I interferon (IFN), in response to bacterial and viral infections, but little is known about a possible induction of type-I IFN in the defense against Candida spp. Here, we show that Candida spp trigger a strong IFN-β response when phagocytosed by primary mouse myeloid dendritic cells (mDCs), through an IFNAR1- dependent activation of intracellular STAT1 as well as IRF7 expression. IFN-β release requires intracellular signaling through Src-family and Syk kinases, although dectin-1 and CD11b play no significant role in the IFN-β release. However, phagocytosis is absolutely required for Cg-induced IFN-β release, since dynasore, a specific inhibitor of dynamin-dependent internalization, completely prevents IFN-β release. MyD88-defficient mDCs do not release IFN-β upon Cg challenge, indicating a phagosomal TLR-dependent mechanism. Strikingly, the IFN-β response requires TLR7, suggesting that fungal nucleic acids may represents PAMPs triggering a type I IFN response. Mouse challenge experiments demonstrate a strong impact of IFN-β signaling on the outcome of experimental candidiasis in vivo. Our work demonstrates for the first time a pivotal role for endosomal TLR7 receptor signaling Candida spp pathogenesis.

461. Trade-offs between wood decay and parasitism: Insights from the genome of the fungal forest pathogen *Heterobasidion irregulare*. Åke Olson[1], Mårten Lind[1], Mikael Branström Durling[1], Magnus Karlsson[1], Igor Grgoriev[2], Andrea Aerts[2], Erika Lindquist[2], Asaf Salamov[2], Susan Lucas[2], Jeremy Schmutz[3], Jane Grimwood[3] Halvor Solheim[4], Carl Gunnar Fossdahl[4], Igor Yakovlev[4], Ari Hietala[4], Francis Martin[5], Annegret Kohler[5], Jan Stenlid[1]. [1]Dept. of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, 75007 Uppsala, Sweden, [2]US DOE Joint Genome Institute, Walnut Creek, California, USA, [3]HudsonAlpha Institute for Biotechnology, Huntsville, Alabama , USA, [4]Norwegian Forest and Landscape Institute, 1432 Ås, Norway, [5]UMR INRA-UHP "Interactions Arbres/Micro-Organismes" IFR 110 "Genomique, Ecophysiologie et Ecologie Fonctionnelles" INRA-Nancy 54280 Champenoux, France.

Parasitism and saprotrophic wood decay are two fungal ecological strategies crucial for succession and nutrient cycling in forest ecosystems, which are rarely possible to study in relation to each other within the same system or organism. The *Heterobasidion annosum sensu lato* (s.l.) species complex is a cosmopolitan pathogen in conifer forests causing massive economic and ecological losses due to tree mortality and wood decay. *H. annosum* (s.l.) provides a possibility for investigating trade-offs between parasitism and saprotrophy. Here we report on the annotated genome sequence of one member of the complex, *H. irregulare*. We identify distinctive parts of the genome that are crucially important for pathogenicity and wood degradation. Global transcriptome analyses show that the expression of the gene set involved in early saprotrophic wood degradation is highly correlated with that of cellulose degradation. In comparison to saprotrophic wood degradation, necrotrophic interaction with pine engages fewer carbohydrate active enzymes, but involves an increase in pectinolytic enzymes as evident from transcriptome analyses combined with mapped quantitative trait loci for pathogenicity. Our results indicate that the energy supply for necrotrophic growth is limited to easily accessible carbohydrates, implying constrained saprotrophic ability in living plant tissue in exchange for access to an additional ecological niche.

462. Identification of secreted *Glomus intraradices* signals activating the plant symbiotic program. Cristina Albarran, Hannah Kuhn and Natalia Requena Plant-Microbe Interactions, Karlsruhe Institute of Technology, Hertzstrasse 16, D-76187 Karlsruhe, Germany. cristina.albarran@bio.uka.de

Arbuscular mycorrhizal (AM) fungi form long-term symbiosis with roots of more than 80% of all land plants and are obligate biotrophs. Similar to other biotrophic fungi colonizing plants, AM fungi need to avoid the defense mechanisms of the plant to develop within the host. A way to achieve this is the delivery of diffusible fungal effectors molecules, termed Myc-factors, which initiate the symbiotic program even before both organisms contact. Although our understanding of the molecular dialogue between AM fungi-host has been improved in the recent years with some clues about the nature of the Myc-factors, still little is know and further investigation is required. In our group, it has been recently shown that some plant genes are specifically induced at early stages by diffusible signals produced by the fungus *Glomus intraradices*. While this activation is partially travelling through the symbiotic transduction pathway (SYM pathway) we have shown that a second cascade is required for the activation of some of those early genes. This suggests that possibly several Myc-factors are secreted at the same time by the fungus. We have established a reporter-assay for the identification and isolation of these Myc-factors. Furthermore, the use of SYM-mutant plant lines will allow distinguish each compound and the signalling cascade that leads to the activation of each gene. Research supported by AvH Foundation and DFG.

463. Effectome comparison of two scab pathogens: *Venturia inaequalis* and *V. pirina*. Kim Plummer[12], Dan Jones[12], Carl Mesarich[34], Joanna Bowen[3], Cecilia Deng[3], Ross Crowhurst[3], Matt Templeton[3]. [1]CRC National Plant Biosecurity, [2]La Trobe University, [3]Plant and Food Research, NZ, [4]University of Auckland. k.plummer@latrobe.edu.au

V. inaequalis and *V. pirina* are hemi-biotrophic fungi that cause apple and pear scab, respectively. Although these two species are closely related and share a similar mode of infection and life cycle, they have a different host range, i.e. *V. pirina* cannot infect apple (*Malus* spp.) and *V. inaequalis* is unable to infect pear (*Pyrus* spp.). We are investigating whether effector recognition is involved in the non-host response. Effectors are secreted pathogen products involved in infection. They can be recognised by plant receptors, initiating a signal transduction cascade that results in resistance. Resistance can be broken if either resistance gene or effector gene is lost or mutated. Effectors often vary across species and races as a consequence of host selection pressure. Effectors are therefore of interest for their roles in infection, in triggering plant resistance, and for differentiating races within a species, or between species. Whole genome sequencing (Illumina), bioinformatics, and proteomics are being used to identify candidate effectors of the two *Venturia* spp. Predicted secretomes and putative effectomes will be compared. Novel effectors will be analysed to determine any role in host-pathogen specificity.

464. Grain from sorghum plants impaired in lignin biosynthesis have reduced colonization by some *Fusarium* species. Deanna Funnell-Harris, Roy French, Scott Sattler and Jeff Pedersen USDA-ARS, Lincoln, NE

Sorghum is commonly infected by multiple members of the *Gibberella fujikuroi* and *Fusarium incarnatum-F. equiseti* species complexes (GFSC and FIESC, respectively). We had developed reduced lignin sorghum lines, usable for bioenergy feedstock, by incorporating two mutations in genes in the lignin biosynthesis pathway: *brown midrib (bmr) 6* and *bmr12*. Grain and leaf tissue from *bmr* and near-isogenic wild-type lines were screened for colonization by *Fusarium*. Isolates from air samples within and above sorghum fields also were collected. One FIESC genotype was absent from *bmr12* grain while the pathogen *Fusarium proliferatum*, in the GFSC, was significantly reduced; both genotypes were common in *bmr6* and wild-type grain and were readily detected in air samples. *Fusarium thapsinum*, another member of GFSC, was the most commonly isolated *Fusarium* from grain, and also infected leaves, but was detected in air at low levels compared with other *Fusarium* species that infect sorghum. To further delineate GFSC and FIESC genotypes, sequences from the histone 3 gene and internal transcribed spacer (ITS), spanning regions 1 and 2, also were analyzed. This work provided evidence that perturbing biosynthesis of lignin affects colonization of sorghum grain by some members of GFSC and FIESC. Evidence from this study also supported the hypothesis that *F. thapsinum* was highly specific to and virulent on sorghum, previously proposed by Leslie and associates.

465. Effector-induced Compatibility of Maize and *Colletotrichum graminicola*. Maria Torres and Lisa Vaillancourt, Department of Plant Pathology. University of Kentucky

Colletotrichum graminicola is an important fungal pathogen of maize. A mutation in the *CPR1* gene, encoding a conserved component of the signal peptidase, led to loss of pathogenicity. When the *cpr1* mutant was inoculated on detached corn leaf sheaths, it germinated and produced appressoria, but usually did not colonize more than the first invaded cell. The mutant was able to colonize sheath tissues that were compromised in host defense. To test the hypothesis that wild-type *C. graminicola* suppresses host defense responses, while the *cpr1* mutant does not, both strains were co-inoculated on detached corn leaf sheaths. Co-inoculation with the wild type allowed the mutant to colonize apparently normally, even when the two strains were inoculated up to 1 cm apart. There was no indication of host cell death, i.e. plant cells surrounding the inoculation sites plasmolyzed and took up neutral red. Preliminary results indicate that one or more factors that induce susceptibility is secreted into culture media by the wild type. Application of culture filtrates from the wild type, but not from the mutant, into the whorl of corn seedlings together with conidia of the *cpr1* mutant, frequently allowed the mutant to colonize and produce anthracnose lesions on susceptible corn seedlings. Our results suggest that *C. graminicola* produces mobile effectors that suppress the ability of host cells to defend themselves against colonization. The *cpr1* mutant is apparently unable to produce these factors, but can take advantage of the favorable environment produced by the wild type.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

466. A novel transcription factor governs opposite- and same-sex mating in the human fungal pathogen *Cryptococcus neoformans.* Marianna Feretzaki and Joseph Heitman Department Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, mf55@duke.edu

Cryptococcus neoformans is a common global human pathogen that causes fungal meningitis in immunocompetent and immunocompromised individuals. Human infection is thought to be caused by inhalation of spores from the environment. The small diameter of spores facilitates alveolar penetration, and following germination the yeast disseminates hematogenously. Nutrient limitation and pheromones induce a dimorphic transition from unicellular yeast to multicellular hyphae during opposite sex mating and unicellular reproduction. Opposite- and same-sex mating are governed by shared components of the conserved pheromone sensing Cpk1 MAPK signaling transduction cascade. However, the homeodomain proteins Sxi1alpha/Sxi2a that regulate sexual reproduction are dispensable for same-sex mating. A recent effort from our lab identified two novel transcription factors, Mat2 and Znf2, which are essential for both opposite- and same-sex mating. In this study we expanded our effort to identify and characterize novel targets of the pheromone signaling cascade. We applied insertional mutagenesis via *Agrobacterium tumefaciens* transkingdom DNA delivery method to identify mutants with a same-sex mating defect. A novel zinc finger transcription factor, named Znf3, was identified through our screen. Znf3 is an essential regulator of hyphal development during opposite- and same- sex mating and plays a key role in cell-cell fusion. Phenotypic and transcriptional analysis provide evidence that Znf3 regulates pheromone production and mating, possibly as a target of the MAPK pathway. Protein-protein and protein-DNA interaction studies will define the mechanism of action of Znf3 and identify its targets.

467. Role of hyphal development in virulence of human fungal pathogen Cryptococcus neoformans Marianna Feretzaki [1], Min Ni[1], Sarah H. Hardison[2], Floyd L. Wormley Jr. [2], and Joseph Heitman[1] [1]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, mf55@duke.edu [2]Department of Biology, University of Texas at San Antonio, San Antonio, Texas

Cryptococcus neoformans is a human fungal pathogen that causes lethal infections of the central nervous system in immunocompromised individuals. In the environment *Cryptococcus* has a defined sexual life cycle with **a** and alpha mating types. Mating leads to the formation of hyphae and spores that are considered to be the infectious propagules of the fungus. Following inhalation, the spores travel to the lung where they establish a pulmonary infection growing as budding yeast. Hyphal development inside the host is rare, possibly due to mammalian physiological conditions. However, our previous work has demonstrated that filamentation and key virulence factors, growth at high temperature and melanin synthesis, are governed by common genetic loci. In our study, we found that a hyperfilamentous strain is hypervirulent compared to the afilamentous, attenuated parental strain using murine and insect models. To further examine the progression of the infection, histological analysis showed that mice infected with the hyperfilamentous strain developed severe lung pathology with collapsed tissue and widespread growth compared to the attenuated parent. We performed linkage analysis to determine whether a single or multiple loci contribute to the observed difference in virulence. The progeny from a cross of the hyperfilamentous strain with the attenuated parent were screened for hyphal initiation and elongation, growth at high temperature and melanization. Murine and insect model hosts will be used to determine virulence of the interesting strains. Our ongoing studies focus on the mammalian innate immune response following infection of hyperfilamentous *C. neoformans*.

468. A metabolomics approach to elucidating the response of wheat to the exposure of *Stagonospora nodorum* effectors. Lauren Du Fall [1] and Peter Solomon[1] [1]Research School of Biology, The Australian National University, ACT, Australia

Stagonospora nodorum is a necrotrophic fungal pathogen of wheat and is the causal agent of Stagonospora nodorum blotch (SNB). This disease is responsible for over $100 million in yield losses in Australia annually. Recent studies have shown that *Stagonospora nodorum* produces a number of proteins (known as effectors) which are suspected to be internalised into host cells of sensitive wheat cultivars. These effectors interact via an unknown mechanism with a dominant wheat sensitivity gene inducing tissue necrosis resulting in disease. The genes encoding three of these effectors, SnToxA, SnTox1 and SnTox3 have been fully characterised. Direct infiltration of these proteins into wheat leaves is sufficient for disease development providing an ideal system in which to study the plant response to these effectors. This research will apply a metabolomics approach to elucidate the cellular processes leading to disease and provide insight into the mode-of-action of these effectors. GC-MS analysis of primary polar metabolites has been undertaken on tissue extracts and apoplastic fluid from ToxA infiltrated wheat, results will be presented. LC-MS analysis of semi-polar secondary metabolites on a Q-ToF instrument will follow in the near future in addition to the analysis of the remaining effectors.

469. Functional analysis of tumor and anthocyanin-inducing effector protein on cluster 19A of *Ustilago maydis*. Shigeyuki Tanaka, Thomas Brefort, and Regine Kahmann. Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany.

Cluster19A is the largest cluster in the genome of *Ustilago maydis*, carrying genes for putative secreted effector proteins. Deletion mutants of the left half of cluster19A (19A_1) show a dramatic reduction of tumor formation and loss of anthocyanin induction, although the mutant retains the ability to grow inside the plant tissue. By generating sub-deletions we show that a group of 5 related genes, $tin1_{1\text{-}5}$, the single genes *tin2* and *tin3* are responsible for the left half deletion phenotype. *tin2* is involved in tumor formation and anthocyanin induction in maize leaves. Introduction of the *tin2* gene into the 19A_1 mutant partially rescued tumor formation and fully restored anthocyanin induction. Tin2 protein lacking the C-terminal 5 amino acids could neither complement tumor formation nor anthocyanin induction. For *tin2* a number of interactors were identified by yeast two hybrid analysis, and one of those is a cytoplasmic maize protein kinase. While this kinase could interact with the full-length Tin2 protein, no interaction could be detected with Tin2 proteins lacking the C-terminal region. We consider it likely that Tin2 is an effector that is translocated to plant cells. Ongoing experiments of co-localization studies of Tin2 and the maize protein kinase will be presented.

470. Role of the F-box protein Fbp1 in protein degradation and pathogenicity of *Fusarium oxysporum*. Cristina Miguel-Rojas, Concepción Hera. Departamento de Genética, Universidad de Córdoba, Spain. E-mail: b02miroc@uco.es

Fbp1 is a non-essential F-box protein of the tomato pathogen, *Fusarium oxysporum* f.sp. *lycopersici*, involved in recruitment of proteins for SCF-ubiquitination and proteasome degradation. The role of Fbp1 in pathogenicity was studied by targeted mutagenesis of the *fbp1* gene. The *fbp1* mutant shows a significant delay in the production of wilt symptoms on tomato plants, and displays several phenotypes related to virulence such as reduced hyphal fusion, root adhesion and inability to penetrate cellophane membranes. To investigate the target proteins recruited by Fbp1, a proteomic approach was carried out. SDS-PAGE and MALDI-TOF-TOF analysis were used to compare proteins in mycelia of the wild-type and *fbp1* mutant strains. We identified 40 proteins differing significantly in abundance between the two strains; 17 of them were more abundant in the *fbp1* mutant strain suggesting a possible regulation by proteasome degradation. RT-PCR analysis was performed in order to distinguish transcriptional and post-transcriptional regulation of the differentially expressed proteins. Our results suggest that Fbp1 regulates protein turnover and pathogenicity in *F. oxysporum*.

471. The way to the root in the *Magnaporthe oryzae*-plant interaction. Raphael Kist, Stephanie Heupel, Karsten Andresen, Eckhard Thines and Natalia Requena Plant-Microbial Interaction, Botanical Institute, Karlsruhe Institute of Technology, Hertzstr. 16, D-76187 Karlsruhe. natalia.requena@kit.edu

Fungal hyphal differentiation during host colonization is an important feature that determines the fate of a fungal-plant interaction. *Magnaporthe oryzae* , a classical leaf pathogen, has been shown to activate a different morphogenetic program, when infecting plants through the root (Dufresne et al., 2001; Sesma & Osbourn, 2004). Interestingly, some of the molecular components required for root infection are shared with other root infecting fungi (Heupel et al., 2010). We address in this project the characterization of the differential hyphal morphogenetic program leading to root colonization. We are employing for that two complementary approaches: a transcriptome profiling comparing root vs. leaf infection using microarray analysis and a screening for root colonization defective insertional mutants. First results from the transcriptomic analysis have revealed that root colonization requires a set of genes apparently specific for root colonization while others are required for growth *in planta* independently of the colonized organ. Among those there is a large set of secreted proteins of unknown function that could be putative effectors. Knock out analysis and *in planta* characterization of the most interesting candidates is in progress.

472. The virulence determinant B-1,3-Gst, Ssw1, plays a pivotal role in wall remodelling during germination in Magnaporthe oryzae. Maeve Price, Marketa Samalova and Sarah Gurr Department of Plant Sciences, University of Oxford. e-mail sarah.gurr@plants.ox.ac.uk

Rice blast fungus Magnaporthe oryzae conidia and germlings undergo a series of morphological changes to effect infection the host plant, culminating in the formation of the appressorium from which a penetration peg is elaborated. We identified a cell wall protein, Ssw1, a member of the Gas family of B-1,3-glycosyltransferases, showing by qRT-PCR analysis that SSW1 is upregulated in the spore, and not during appressorium formation. Mutant ssw1 strain is significantly less virulent than the wild-type strain, leading us to speculate and demonstrate that attenuated virulence is due to defects in the transition between filamentous and isotropic growth, so impairing penetration peg formation. Consistent with this, microscopic observation of the uptake of labelled oligosaccharides suggests that the knockout of SSW1 inhibits the incorporation of B-1,3-glucan into the cell wall. Nanoscale atomic force microscopy observation of the cell wall surface shows that deletion of SSW1 results in a reduction in the diameter of putative B-1,3-glucan fibrils, present on the surface of conidia. Furthermore, the ssw1 mutant conidium cell wall is significantly less elastic than the wild-type strain cell wall. We will report on these findings together with GFP localisation assays and data showing that B-1,3 glucan shed by the mutant strain elicits a defence response in the host plant.

473. Assessing host factors involved in Cryptococcal infection. Deepa Srikanta, Matthew Williams, & Tamara L. Doering Washington University Medical School, Dept. of Molecular Microbiology srikanta@borcim.wustl.edu

Cryptococcus neoformans is a fungal pathogen that causes serious disease in immunocompromised mammalian hosts. Cryptococcosis is contracted by the inhalation of infectious particles, leading to a primary pulmonary infection. As disease progresses, the fungus disseminates to the brain, causing lethal meningitis. Along with cryptococcal virulence traits, host phagocytic factors are involved in pathogen latency and dissemination. Our goal in this study is to identify host factors involved in the interaction of phagocytic cells with *C. neoformans*, and understand the relevant mechanisms. This will increase our knowledge of microbial pathogenesis, and may help to identify potential therapeutic targets. We used RNA interference (RNAi) to reduce gene expression in host phagocytic cells and applied high- throughput methods to study the effects of this on uptake and adherence of *C. neoformans*. An initial screen of human phosphatase and kinase RNAi libraries identified 35 potential host factors that significantly affect adherence and uptake. We are now verifying and characterizing these candidates. Results from optimization of the screen and follow-up studies of initial hits will be presented. Our results suggest that this is a productive approach, which can be expanded to additional assays and broader RNAi libraries to elucidate the complex relationships between hosts and pathogens at the genome level. This research is supported by NIH AI082004. D.S. is supported by NIH Training Grant 5T32AI007172.

474. Global analysis of the evolution and mechanism of echinocandin resistance in a series of *Candida glabrata* clinical isolates. Sheena D. Singh-Babak & Leah E. Cowen Department of Molecular Genetics, University of Toronto, Ontario, Canada

Candida species are the leading fungal pathogens of humans and *C. glabrata* is now second to *C. albicans* as the most prevalent *Candida* species due to its intrinsic resistance to the most widely used class of antifungals, the azoles. As a result, the newest class of antifungals, the echinocandins, is commonly employed to treat *C. glabrata* infection. My work thus far established that the molecular chaperone Hsp90 plays a role in resistance to the cell wall stress exerted by the echinocandins via the client protein calcineurin in *C. albicans*. Here we present new work that implicates both Hsp90 and calcineurin as regulators that enable survival of cell wall stress exerted by echinocandins in a series of *C. glabrata* isolates that evolved drug resistance in a human host. Genome wide sequencing unveils 45797 single nucleotide variants between the latest clinical isolate and the reference sequence CBS138. Strikingly, only 9 non-synonymous SNVs between the early and late clinical isolates were found. Furthermore, we find a mutation in the echinocandin target FKS2 previously reported to confer resistance in *C. glabrata* clinical isolates. Quantitative RT-PCR experiments revealed that deletion of calcineurin blocks the induction of the resistance determinant FKS2. Thus, our work identifies mutations that accompany the evolution of drug resistance in a human host on a genome-wide scale and suggests a new mechanism of resistance to the echinocandins.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

475. Mutation of the *Ustilago maydis* ortholog of the *Aspergillus nidulans* sporulation regulator *medA* affects mating and pathogenicity. Nadia Chacko and Scott Gold, Department of Plant Pathology, University of Georgia, Athens, GA

Corn smut caused by *Ustilago maydis* is an important model system in the study of obligate plant pathogenesis. Mating of yeast cells of opposite mating type initiates infection. The resulting dikaryotic filaments proliferate inside the host producing galls inside which dark diploid teliospores are produced. For the study of sporulation in *U. maydis*, comparison with *Aspergillus nidulans*, a model organism with a well-characterized sporulation pathway, is useful. In *A. nidulans*, a transcription factor, *medA*, regulates several factors of the central conidiation network and mating genes *matA* and *matB*. The ortholog of *medA*, was identified in *U. maydis* by searching its genome. This gene, *med1* (um03588), was deleted using the DelsGate technique, in several genetic backgrounds. The mutants were defective in mating *in vitro* and reduced in pathogenicity. Solopathogenic strains were also reduced in pathogenicity and were non-filamentous on YPD-charcoal mating plates indicating an additional post fusion role for the gene in *U. maydis*. Drop mating assays showed that the mutant produces conjugation tubes although fusion was not observed. Real time PCR showed that the expression of genes encoding the mating pheromone and receptor were down-regulated in the mutant. The expression of transcription factor *prf1*, that regulates these mating related genes, was also down-regulated in *med1* mutants indicating *med1* may be upstream of *prf1*.

476. The genome of the obligate endobacterium of an AM fungus reveals an interphylum network of nutritional interactions. S. Ghignone˚, A. Salvioli˚, I.Anca˚, E. Lumini˚, S.Cruveiller§ G.Ortu˚, P.Piffanelli*, L. Lanfranco˚ and P. Bonfante˚ ˚ Department of Plant Biology, University of Torino and IPP-CNR, Italy § CEA Institut de Génomique – Genoscope, Paris France * Parco tecnologico Padano, Lodi - Italy

Many AMF host endobacteria in their cytoplasm. To elucidate the role of the Gram negative endobacterium Candidatus Glomeribacter gigasporarum (CaGg), we sequenced its genome using a metagenomic approach which combined Sanger sequencing of fosmid clones from a Gigaspora margarita (the host) library with a 454 pyrosequencing of an enriched- endobacterial fraction. The final assembly led to 35 contigs, totalling 1.72 Mb, consisting of a chromosome and three plasmids. The genome features of CaCg place it in the Burkolderiaceae group, while metabolic networks analysis clustered CaCg with insect endobacteria, mirroring its obligate intracellular life-style. CaCg resembles an aerobic microbe, with no fermentative and limited energy-production capabilities via glycolysis and phosphate-pentose pathways. It depends upon its fungal host for C, P and N supply; its ability to synthesize amino acids is limited suggesting that most amino acids are imported from its host. In summary, the bacterial genome data of the first endosymbiont's endosymbiont revealed a novel context of intimate symbiosis between bacteria and fungi. Since the CaGg fungal host is itself an obligate biotroph dependent on its photosynthetic host, our work uncovers a network of nutritional/evolutionary interactions between plants, AM fungi and endobacteria.

477. A Yeast Recombination-Based Cloning System for High-Throughput Gene Analyses in *Botrytis cinerea*. Julia Schumacher Institut für Biologie und Biotechnologie der Pflanzen, Westf. Wilhelms-Universität,Schlossgarten 3, 48149 Münster, Germany; jschumac@uni-muenster.de .

B. cinerea is the causative agent of grey mould disease in more than 200 plant species. Because of its worldwide importance, the underlying mechanisms of the infection process are extensively studied. An important aspect of molecular genetics of *B. cinerea* is the high efficiency (70–100%) of targeted gene inactivation, which allows a rapid functional analysis of putative pathogenicity-related genes. However, molecular tools basing on the expression of reporter gene constructs are hampered by weak expression levels and insufficient fluorescence of the reporters when conventional expression vectors are used. Hence, we have initiated an approach to establish an expression system for *B. cinerea* regarding the following aspects: (i) the targeted integration of the constructs at defined gene loci which are dispensable under standard conditions, (ii) the use of promoter and terminator sequences allowing optimal gene expression, (iii) the use of modified reporter genes, (iv) the use of different selection markers, and (v) the choice of a highly efficient cloning system. We demonstrate the successful application of the expression system for labeling the cytosol, nuclei, and membranes by fusing reporter genes with selected *B. cinerea* genes. In addition, vectors containing fragments of the modified *gfp* for BiFC analyses have been generated and proved for their suitability in *B. cinerea*.

478. Identification of New Virulence Factors by T-DNA-mediated Insertional Mutagenesis in *Botrytis cinerea*. Julia Schumacher, Sabine Giesbert, and Paul Tudzynski Institut für Biologie und Biotechnologie der Pflanzen, Westf. Wilhelms-Universität, Schlossgarten 3, 48149 Münster, Germany; jschumac@uni-muenster.de .

By using an *Agrobacterium tumefaciens*-mediated transformation (ATMT) approach in the grey mold fungus *B. cinerea* we generated a library with 2,350 transformants carrying random integrations of a hygromycin resistance cassette flanked by LB and RB sequences. By performing Southern blot analyses of randomly chosen transformants it was shown that most of the ATMT strains contain single copies of the T-DNA. A first virulence screen of all transformants on detached tomato leaves resulted in the identification of 560 less virulent strains. 231 of these have been undergone a second screening on primary leaves of *Phaseolus vulgaris*, and the less virulent phenotype has been confirmed for 169 strains. Up to now, the T-DNA insertion sites in 75 transformants have been identified by TAIL-PCR analyses, illustrating that T-DNA integrations preferably occur in non-coding regions. Twelve genes tagged by a single T-DNA insertion either in the coding or non-coding region have been chosen to validate the procedure. So far, knock-out approaches for eight candidate genes have been completed successfully: six knock-out strains are impaired in virulence on *P. vulgaris* as observed for the corresponding ATMT mutants; for two genes, the confirmation of the ATMT phenotype has failed. Thus, the screening of an ATMT library is suitable to identify new genes involved in the *B. cinerea* – host interaction.

479. Genome sequencing of *Fusarium pseudograminearum* reveals a horizontally transferred amidohydrolase involved in virulence. Donald M. Gardiner[1], Peter Solomon[2], Megan McDonald[4], Mhairi Marshall[3], Kemal Kazan[1], Sukumar Chakraborty[1], Bruce McDonald[4], John M. Manners[1] [1]CSIRO Plant Industry, Brisbane, Queensland 4067, Australia. [2]Research School of Biology, The Australian National University, Canberra ACT 0200, Australia. [3]Queensland Facility for Advanced Bioinformatics, Queensland 4072, Australia. [4] Institute of Integrative Biology, ETH Zurich, LFW, Universitaetstrasse 2, CH-8092 Zürich, Switzerland Donald.Gardiner@csiro.au

Fusarium crown rot disease is a chronic problem in wheat and barley in arid environments such as Australia and there are currently no fully resistant wheat cultivars. In Australia, *F. pseudograminearum* is the pathogen predominantly associated with crown rot although related Fusarium species such as *F. graminearum* and *F. culmorum* have the ability to cause crown rot in some regions. These three *Fusarium* species can also cause globally important head blight disease of wheat. To increase our understanding of factors affecting pathogen virulence, we have sequenced the genome of an *F. pseudograminearum* isolate and compared it to the publically available genome sequence of *F. graminearum*. Despite overall sequence conservations, striking differences have also been observed between the genomes of these two *Fusaria*, including the presence of completely novel secondary metabolite gene clusters. Most strikingly we also identified a gene encoding an amidohydrolase that appears to have been acquired by horizontal gene transfer. This gene has a clear orthologue in the genome of the wheat pathogen *Stagonospora nodorum* but not in any other fungal genome and the next closest sequence matches are from bacteria. Deletion of this gene from *F. pseudograminearum* resulted in a reduction in virulence on barley but not wheat. We are currently undertaking sequence analysis of this gene in populations of both species to determine its origin. Its presence in these two unrelated pathogens but not in any other fungal species suggests a role for this gene in a common pathogenesis mechanism that targets an important defence pathway in cereals.

480. A subset of the secretome of *Venturia inaequalis* is highly up-regulated during infection of apple. Joanna Bowen[1], Carl Mesarich[1,2], Wendy Kearns[1,2], Cecilia Deng[1], Ross Crowhurst[1], Jason Shiller[3], Erik Rikkerink[1], Kim Plummer[3], Matthew Templeton[1]. [1]The New Zealand Institute for Plant and Food Research, Auckland, New Zealand. [2]The University of Auckland, Auckland, New Zealand. [3]La Trobe University, Melbourne, Australia.

The pathogen *Venturia inaequalis* infects members of the Maloideae, causing the disease apple scab. The genetics of the interaction between *Malus* and *V. inaequalis* follow the gene-for-gene model; effectors (pathogen proteins required for infection) are presumably secreted into the plant/pathogen interface early in the infection cycle. The whole genome sequence of isolate MNH120 (race 1) was determined using Illumina technology. A total scaffold length of 48Mb was assembled at an estimated 96% coverage of genes calculated by mapping 131 SSR markers and ESTs. Gene predictions using Genemark™ detected 12,313 putative open reading frames, including 2,186 with a predicted secretory leader sequence (the secretome), of which 382 were novel. Analysis of the *in planta* transcriptome revealed a subset of these were the most highly up-regulated during infection, making them strong effector gene candidates. In addition, orthologues of several fungal effector genes, including *Ecp6* and *Avr-Pita*, and a gene family with similarity to *AvrLm6*, were identified. Functional characterisation of these genes is currently being carried out.

481. Siderophore utilization in *Cryptococcus neoformans*. Sanjay Saikia and James Kronstad Michael Smith Laboratories, University of British Columbia, Vancouver V6T1Z4, Canada.

The fungus *Cryptococcus neoformans* is a major human pathogen that causes life-threatening meningoencephalitis. Within the host the fungus faces several challenges for growth and proliferation including limited iron availability. However, like other pathogenic fungi, *C. neoformans* possesses several iron acquisition mechanisms, including reductive iron uptake, siderophore uptake and utilization of both heme and transferrin. Although the reductive iron uptake system has been characterized at the molecular level in *C. neoformans*, detailed studies on other iron uptake systems are lacking. Recently, we found that HapX, the regulatory subunit of the CCAAT-binding complex, has a positive regulatory role for a subset of genes encoding siderophore transporters. However, *C. neoformans* is not known to produce siderophores although the fungus has at least seven siderophore transporters. In our recent studies, we found that the fungus is able to utilize xenosiderophores as iron source. In this context, we are interested in understanding the role of siderophore-mediated iron uptake system in cryptococcal growth and disease development.

482. Structural analysis and gene expression profiling of the 1.4-Mb chromosome encoding the AM-toxin biosynthetic gene cluster in the apple pathotype of *Alternaria alternata*. Yoshiaki Harimoto[1], Megumi Kawase[1], Motoichiro Kodama[2], Mikihiro Yamamoto[3], Hiroshi Otani[2], and Takashi Tsuge[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya University,Japan. [2]Faculty of Agriculture, Tottori University, Japan. [3]Faculty of Agriculture, Okayama University, Japan. harimoto@agr.nagoya-u.ac.jp

The apple pathotype of *Alternaria alternata* produces host-specific AM-toxin and causes Alternaria blotch of apple. Previously, we identified four AM-toxin biosynthetic genes (*AMT1* to *AMT4*) and putative *AMT* cluster (70 kb) consisting of 17 genes. Here, we analyzed the nucleotide sequence of the 1.4-Mb chromosome of strain IFO8984, which encodes *AMT* cluster, using Genome Sequencer 20 System and obtained 24.2-Mb sequences (redundancy = 17.3) assembled into 47 contigs (822.7 kb, 58.8% coverage). The sequence analysis of 17 BAC clones and the optical mapping analysis of the chromosome made 4 contigs (1147 kb, 81.9% coverage). Mapping of *AMT* genes revealed that IFO8984 has multiple sets of *AMT* clusters on one side of the chromosome. Structuresof multiple sets of the *AMT* clusters were highly conserved, but not identical. We also analyzed the transcription levels of all genes, which were mapped on the 1.4-Mb chromosome, in AM-toxin producing and non-producing cultures by real-time quantitative RT-PCR. This analysis showed that the transcription levels of most genes were markedly lower than that of *EF1- alpha* used as a control in both cultures. We found some genes upregulated in the toxin producing culture as candidates of new *AMT* genes.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

483. The Fusarium virguliforme gene *fvgrx2* affects the tolerance of the fungus to the soybean phytoalexin glyceollin. Mansouri, Saara, and Ahmad M. Fakhoury. Southern Illinois University Carbondale, IL, USA.

Fusarium virguliforme is a soil-borne pathogen that causes Sudden Death Syndrome (SDS) in soybean. Although SDS is a very destructive disease, little is known about the fungal processes that lead to its development. Soybeans produce and accumulate phytoalexins as a mechanism of defense against invading plant pathogens. One of the major phytoalexins produced is Glyceollin. In vitro assays show that glyceollin has a fungicidal effect on *F. virguliforme*. Transmission electron microscopy and fluorescence microscopy reveal that the exposure of *F. virguliforme* to glyceollin affects the integrity of the fungal cell plasma membrane. The *F. virguliforme* gene *fvgrx2* transcripts consistently show elevated levels of accumulation when the fungus is exposed to glyceollin. *Fvgrx2* encodes a glutaredoxin. Glutaredoxins are small molecules usually induced in cells in response to stresses including oxidative stress. *Fvgrx2* was disrupted in *F.virguliforme*. The *delta Fvgrx2* transformant was hypersensitive to oxidative stress. Upon challenging soybean plants with the fungal transformant, the disruption of *fvgrx2* resulted in a significant decrease in the ability of the fungus to infect and colonize the roots of the challenged plants. A yeast two-hybrid screen was also used to identify FvGRX2 interacting partners. The work presented here uncovers a role of *fvgrx2* in affecting the aggressiveness of *F. virguliforme* on soybean by aiding the fungus in overcoming the effect of glyceollin.

484. Withdrawn

485. *In vitro* production of neutrophil extracellular traps against *Aspergillus fumigatus* is influenced by the conidial surface protein hydrophobin RodA. Sandra Bruns[1,2], Mike Hasenberg[3], Olaf Kniemeyer[1,2], Andreas Thywißen[1,2], Vishukumar Aimanianda[4], Jean-Paul Latgé[4], Matthias Gunzer[3], Axel Brakhage[1,2] [1]Department of Molecular and Applied Microbiology, Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute (HKI) – Jena, Germany [2]Department of Microbiology and Molecular Biology, Friedrich-Schiller-University-Jena, Germany [3] Institute for Molecular and Clinical Immunology, Otto-von-Guericke-University, Medical Faculty, Magdeburg, Germany [4] Unite des Aspergillus, Institue Pasteur, Paris F-75015, France

Aspergillus fumigatus is the most important airborne fungal pathogen causing life-threatening infections in immunocompromised patients. Conidia as the infectious agent infiltrate the lungs and get in contact with alveolar macrophages and neutrophil granulocytes, which repesent the first line of defense. These cells kill fungal conidia by phagocytosis. Neutrophils are also able to form neutrophil extracellular traps (NETs) against *A. fumigatus* conidia and hyphae. These sticky filaments consist of nuclear DNA decorated with histones and fungicidal proteins. Time-lapse movies of coincubations of *A. fumigatus* with neutrophils revealed that NET production was a highly dynamic process which, however, was only exhibited by a sub-population of cells. In addition the intensity of NET formation by unstimulated, human neutrophils was strain- and morphotype-dependent. The killing of *A. fumigatus* conidia was not influenced by the amount of released extracellular DNA, but metabolic activity of hyphae seemed to be reduced by NETs after longer incubation periods of 12h. Our data suggest that NETs prevent further spreading, but apparently do not represent the major factor for killing. By using fungal mutants and extracted RodA protein we demonstrate that the conidial hydrophobin RodA, a surface protein rendering conidia immunologically inert, led to reduced NET formation by neutrophils encountering *Aspergillus*. We are currently investigating NET formation against different *A. fumigatus* mutants to identify fungal components, which stimulate or reduce NET formation. Bruns et al.(2010) Plos Pathogens 6:e1000873 Brakhage, A.A., S. Bruns, A. Thywissen, P. F. Zipfel, J. Behnsen(2010) Curr Op Microbiol 13:409

486. New insights in the regulation of mycotoxin production by the plant pathogen *F. graminearum*. Jörg Bormann*, Peter Ilgen, Cathrin Kröger, Birgit Hadeler and Wilhelm Schäfer Biozentrum Klein Flottbek | - Molecular Phytopathology and Genetics - | Ohnhorststr. 18 | 22609 Hamburg, Germany | Tel.: +49-40-42816309 | Fax: +49-40-42816357 | bormannj@botanik.uni-hamburg.de

The fungal pathogen *Fusarium graminearum* is the causal agent of Fusarium head blight in small grain cereals and of cob rot disease of maize. The devastating effect is due to yield losses and mycotoxin contamination. Among the mycotoxins produced by the fungus, the trichothecene deoxynivalenol (DON) was shown to be important for virulence in wheat. The regulation of DON-production during plant infection and in axenic culture is still not known in detail. Using qRT-PCR and Elisa-based DON measurements we analyse the influence of different nitrogen sources and plant substances on a) DON production and b) on genes that play a certain role in nitrogen signaling. These analyses are accompanied by fluorescence measurements using a reporter strain that expresses the green fluorescent protein GFP under the control of the trichodien synthase (Tri5) promoter. Using this strain it is possible to directly monitor Tri5 induction under different growth conditions. We show a DON-inducing effect of ammonium ions and plant components. In addition, we started to functionally analyse nitrogen signaling regulator proteins like the GATA-transcription factor AreA and the bZIP-transcription factor MeaB in order to assess their function in the regulation of toxin production and virulence.

487. Fungus-plant interaction in MEK knock out mutants of *Cryphonectria parasitica.* Massimo Turina and Marino Moretti IVV-CNR, Str. delle Cacce 73, 10135 Torino, Italy

We are currently investigating the biological function(s) of *Cryphonectria parasitica cpkk1*, *cpkk2* and *cpkk3* genes, encoding the three mitogen-activated protein kinase kinase (MEK) present in the *C. parasitica* genome. Cpkk1 is a MEK putatively acting in a phosphorylation cascade essential for cell integrity; Cpkk2 is the *C. parasitica* homologue of Ste7 from *Saccharomyces cerevisiae* involved in pheromone-responsive pathway and Cpkk3 is the *C. parasitica* homologue of *S. cerevisiae* Pbs2, a MEK involved in high osmolarity growth response. Knock out mutants were obtained for each of the three MEKs. While *cpkk1* null-mutant phenotypical characterization is still under investigation, *cpkk3* null-mutant is indistinguishable from wild type except from a markedly reduced growth in high osmolarity media. Growth defect, abolished conidiation and sporulation and reduced pigmentation were observed in *cpkk2* null-mutant strain. For this mutant, a dramatic reduction in virulence was also detected in pathogenicity tests on apple fruit and chestnut (*Castanea sativa*) stands. No influence on laccase activity was observed, while an up-regulation of the pheromone gene *Mf2/1* was detected. We were unable to transmit CHV1 hypovirus infection to *cpkk2* null-mutant through anastomosis. Due to these important outcomes, a proteomic approach was considered in order to study qualitative and quantitative protein expression levels in *cpkk2* k.o. strain. A comparative analysis between the protein profiles of this mutant and the wild-type strain during growth in presence and absence of chestnut wood are currently under investigation.

Published by New Prairie Press, 2017

488. The bZIP transcription factor HapX controls iron homeostasis and virulence of *Fusarium oxysporum* on plant and mammalian hosts. Manuel S. López-Berges[1], Javier Capilla[2], Emili Mayayo[2], Christoph Jöchl[3], Sandra Matthijs[4], Josep Guarro[2], Pierre Cornelis[4], Hubertus Haas[3] and Antonio Di Pietro[1]. [1]Departamento de Genética, Universidad de Córdoba, Spain. [2]Unitat de Microbiologia, Facultat de Medicina, Universitat de Tarragona, Spain. [3]Division of Molecular Biology, Biocenter, Innsbruck Medical University, Austria. [4]Flanders Interuniversity Institute for Biotechnology, Vrije Universiteit Brussels, Belgium.

The soilborne fungus *Fusarium oxysporum* causes vascular wilt disease on more than a hundred plant species and opportunistic infections in humans that range from superficial or locally invasive to disseminated with mostly lethal outcomes. Previous work established that a single isolate of *F. oxysporum* f. sp. *lycopersici*, FGSC 9935, can cause disease on both tomato plants and on immunodepressed mice. Here we have studied the role of the bZIP protein HapX, a key regulator of iron homeostasis whose transcription is strongly induced under iron-depleted conditions. As previously reported in *Aspergillus*, deletion of *hapX* in *F. oxysporum* causes derepression of key genes involved in iron-dependent pathways such as *sreA* (repressor of siderophore metabolism), *cycA* (cytochrome C, respiration), *hemA* (α-amino-levulinic acid synthase, heme biosynthesis), *acoA* (aconitase, TCA cycle) or *lysF* (homoaconitase, lysine biosynthesis), leading to impaired growth under iron- depleted conditions. *F. oxysporum* mutants lacking *hapX* showed a delay in the induction of vascular wilt symptoms on tomato plants. Moreover, mortality and fungal load of immunodepressed mice infected with the *hapX* mutant strain was drastically reduced compared with the wild type and the complemented strains. Our results suggest that the function of HapX is conserved between *Fusarium* and *Aspergillus* and that iron homeostasis is crucial for pathogenicity of *F. oxysporum* both on plants and on mammals.

489. Crystal Structure Of The Avirulence Gene *AvrLm4-7* Of *Leptosphaeria maculans* Illuminates Its Evolutionary And Functional Characteristics. I. Fudal[1], F. Blaise[1], K. Blondeau[2], M. Graille[2], A. Labarde[2], A. Doizy[2], B.M. Tyler [3], S.D. Kale[3], G. Daverdin[1], M.H. Balesdent[1], H. van Tilbeurgh[2] and T. Rouxel[1 1] INRA-Bioger, Grignon, France [2] IBBMC-CNRS / Université Paris-Sud, Orsay, France [3] Virginia Bioinformatics Institute, Blacksburg, USA

Leptosphaeria maculans, an ascomycete causing stem canker of oilseed rape, develops “gene-for-gene” interactions with its host plant where fungal avirulence (*AvrLm*) genes are the counterpart of plant resistance (*Rlm*) genes. *AvrLm4-7* encodes a 143 aa cysteine-rich protein, potentially secreted, strongly induced during primary leaf infection and involved in fungal fitness. AvrLm4-7 is translocated into plant and animal cells. This translocation is mediated by binding to PI-3-P and necessitates the presence of a RxLR-like motif. AvrLm4-7 crystal structure was determined following heterologous production in *Pichia pastoris*. The protein shows the presence of 4 disulfide bridges and is strongly positively charged, suggesting interaction with minus charged molecules (DNA, phospholipids). AvrLm4-7 confers a dual specificity of recognition by *Rlm7* or *Rlm4* resistance genes and occurs as three alleles only: the double avirulent (A4A7), the avirulent towards *Rlm7* (a4A7), or the double virulent (a4a7). A unique event of mutation, leading to the change of a glycine residue to an arginine, an amino acid located on an external loop of the protein, is responsible for the A4A7 to a4A7 phenotype change, strongly suggesting the importance of this protein region for recognition by the *Rlm4* gene, but not for the effector function of *AvrLm4- 7*.

490. Characterization of a cluster of genes required for pathogenic development of *Ustilago maydis* in maize. Jinsong Wu and Regine Kahmann Dept. Organismic Interactions, Max-Planck-Institut für terrestrische Mikrobiologie, Karl-von-Frisch-Strasse, 35043 Marburg, Germany wuj@mpi-marburg.mpg.de

Ustilago maydis is a biotrophic basidiomycete fungus causing smut disease in maize. Genome analysis has revealed the existence of large number of secreted proteins with unknown function, many of which are arranged in gene clusters. Here we investigate the role of one these gene clusters (cluster 10A), which includes 10 genes. Deletion of the whole cluster in the solopathogenic strain SG200 results in significantly reduced virulence (Kämper et al., 2006). By generating sub-deletions we have identified the gene Um03744 as the major gene responsible for the virulence in this cluster, and this result was confirmed by re-introducing the native gene back to the deletion mutant. The transcript of Um03744 is not detectable in sporidia but dramatically induced after infection in maize leaves after 2 and 4 day post infection. Deletion of um03744 only does not influence the growth of fungus in liquid axenic culture, but affects its virulence significantly. The reduced virulence is associated with less fungal biomass in infected leaf tissues. Under the control of a constitutive promoter, the Um03744 protein is secreted in SG200 in axenic culture, but with smaller size than expected, suggesting that the protein is processed during secretion. Deletion analysis revealed that only the central domain of Um03744 is essential for the virulence. The analysis of JA, SA, and ABA in leaves infected with SG200 and Um03744 deletion strains revealed the reduced levels of JA and ABA after infection of the deletion mutant, suggesting that Um03744 affects host defense responses.

491. Tomatinase Tom2 is required for full virulence of *Fusarium oxysporum* on tomato plants. N. Gómez-Muñoz, C. Ruiz-Roldán and M. I. G. Roncero* University of Cordoba, Spain.*E-mail: ge1gorom@uco.es

Tomato plants produce the antifungal compound α-tomatine, a steroidal glycoalkaloid consisting of an aglycone moiety (tomatidine) and an oligosaccharide moiety (β-lycotetraose) composed of four sugars. *Fusarium oxysporum* f. sp *lycopersici*, a tomato pathogen, produces the tomatinase enzyme Tom1, which degrades α-tomatine to less toxic derivates. Tom1, although not essential for pathogenicity, is required for the full virulence of this fungus. *F. oxysporum* genome contains four additional putative tomatinase genes (*tom2* to *tom5*) with identities to tomatinases from family 3 of glycosyl hydrolases, which might contribute to the complete hydrolysis of α-tomatine. The aim of this study was to explore the role of *tom2* in the pathogenic behavior of *F. oxysporum*. By target-directed transformation we generated deleted Δ*tom2* mutants that showed slightly higher sensitivity to α-tomatine than the wild type strain, and 45% reduction in total tomatinase activity when compared to the wild type strain. Tomato plants infected with the knockout mutants showed a significant delay in the disease process, indicating that Tom2, although not essential for pathogenicity, is required for the full virulence of *F. oxysporum.* Our results indicate that detoxification of α-tomatine in *F. oxysporum* is carried out by the concerted action of several tomatinase activities, suggesting the importance of these enzymes during the infection process. In order to determine the mechanism of action of these tomatinases, we are currently performing HPLC-MS analysis of the hydrolysis products of α-tomatine released by the heterologously expressed, in *Escherichia coli*, Tom1, Tom2 and Tom5. Simultaneous inactivation of the tom genes should help to unravel the intricacies of the pathogenicity process in *F. oxysporum.*

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

492. Characterization of Bem1 in *Botrytis cinerea* and *Claviceps purpurea*. Janine Schürmann, Sabine Giesbert, Leonie Kokkelink and Paul Tudzynski Westfälische Wilhelms-Universität, Institute for Biology and Biotechnology of Plants, Schlossgarten 3, D-48149 Münster, Germany j_schu51@uni-muenster.de

Scaffold proteins are critical for the localization and binding of diverse signaling components; one well-known scaffold is Bem1 that is known to be involved in polarity establishment in *S. cerevisiae*. In this organism Bem1 plays a major role in the formation of a complex bringing together the small GTPase Cdc42, the GEF Cdc24 and the PAK kinase Cla4 at sites of polar growth. Homologues of Bem1 have been identified in the plant pathogenic fungi *Botrytis cinerea* and *Claviceps purpurea*. Currently, we are analyzing the function of this scaffold protein during infection, comparing the effects on necrotrophic and biotrophic lifestyles. The Δ*bem1* mutants of *B. cinerea* form round shaped conidia that show an altered germination behavior and are impaired in plant surface penetration. Interestingly, the phenotype partially overlaps with that of Δbc*cdc42* strains; thus, an interconnection between these two proteins can be assumed. Protein interaction studies of *C. purpurea* clearly show an interaction between CpBem1 and CpCla4. Moreover, the phenotype of Δcp*bem1* mutants points towards the drastic phenotype of Δ*cla4* and Δ*rac* strains which form cauliflower-like colonies obviously being disturbed in polar growth. Unexpectedly, the conserved scaffold protein Bem1 seems to fulfill different functions within these two plant pathogenic fungi.

493. Role of N-acetylglucosamine transferases in *Fusarium oxysporum* virulence. L. López-Fernández[*1], A. Prieto[2], C. Ruiz-Roldán[1], M. I. G. Roncero[1]. [1]University of Cordoba, Spain; [2]CIB-CSIC, Madrid, Spain, [*]Email: z92lofel@uco.es

The cell wall is a vital rigid structure formed by an inner complex layer of chitin and glucan microfibrils, and an outer glycoproteins layer bound to the polymers. The functionality of protein glycosylation in pathogenic fungi is multiple, complex and not well understood, nonetheless, it has been suggested to play important biological roles in cellular recognition events and correct protein folding involving extracellular enzyme activity. Glycosyltransferases are residents of the membranes lining the ER, the Golgi apparatus and the trans-Golgi network. They act sequentially, so that the glycosylated product of one reaction becomes the acceptor substrate for the next. In this way, long and complex linear or branching oligosaccharides are built. In silico analysis revealed that *Fusarium oxysporum* genome contains seven putative N- acetylglucosaminetransferase genes (*gnt*). By target-directed transformation, deleted and constitutively expressed *gnt* mutants were obtained. Both types of mutants showed dramatic reduction of the infection capacity on tomato plants. Defective mutants displayed normal vegetative growth and spore formation, as well as growth inhibition on SDS or Calcofluor-White. Total polygalacturonase activity in defective mutants was decreased, under induction conditions, in comparison to wild type or constitutive strains. Chemical analysis of cell walls showed that the main component in all strains is the β-glucan-chitin complex. In addition, the wild type contains significant amounts of N- and O-linked α-(1,4)-glucans and minor quantities of acidic galactomannans. Defective *gnt* mutants revealed significant reduction of N-linked glycans, but no variation in the level of O-linked glycans. Chemical characterization of the polysaccharidic material from both types of mutants is currently under study.

494. Random T-DNA insertion mutagenesis in *Verticillium dahliae* to identify novel pathogenicity genes. Parthasarathy Santhanam, and Bart P H J Thomma, Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands. partha.santhanam@wur.nl

Vascular wilt diseases caused by soil-borne pathogens are among the most devastating plant diseases worldwide. The fungus *Verticillium dahliae* causes vascular wilt diseases in over 200 dicotyledonous species. An effective way to identify genes that are required by the pathogen to cause disease on its host(s) is random mutagenesis followed by pathogenicity testing. *Agrobacterium tumefaciens*-mediated gene transfer (ATMT) has been widely used for large scale insertional mutagenesis in fungi, where the Agrobacterium transfers a part of its plasmid (T-DNA) into the host genome. Resulting mutants can be analyzed for altered pathogenicity or virulence. As mutants are tagged by the T-DNA, subsequent cloning of the gene of interest is possible. Through ATMT, we have generated 900 random insertional mutants in *V. dahliae* that are analyzed for reduced pathogenicity or virulence on susceptible tomato. This has resulted in the identification of 80 transformants that are severely compromised in pathogenicity. The T-DNA integration site of the selected transformants were identified using RSE-PCR and functional characterization of the respective genes will be carried out to identify their contribution to *Verticillium* wilt.

495. The oomycete RxLR-effectors AVR3a and SpHtp1 show cell type specific import and their RxLR-leaders mediate dimerisation. Stephan Wawra[1], Severine Grouffaud[1,2], Judith Bain[1], Anja Matena[3], Claire Gachon[4], Irene de Bruijn[1], Stephen Whisson[2], Peter Bayer[3], Paul Birch[2], Pieter van West[1] [1] Aberdeen Oomycete Laboratory, Aberdeen (UK) [2] Scottish Crop Research Institute, Dundee (UK) [3] Universität Duisburg-Essen, Essen (Germany) [4] Scottish Association for Marine Science, Oban (UK)

The fungus-like oomycetes contain several species that are devastating pathogens of plants and animals. During infection oomycetes translocate effector proteins into host cells where they interfere with host defence responses. Several oomycete effectors have a conserved Arg-Xaa-Leu-Arg (RxLR)-motif that is important for their delivery. We found that, whereas the RxLR-leader sequence of SpHtp1 from the fish pathogen *Saprolegnia parasitica* shows fish cell- specific translocation, the RXLR-leader of AVR3a from the potato-late-blight pathogen *Phytophthora infestans* promotes efficient binding of the C-terminal effector domain to several cell types. Our results demonstrate that the RxLR- leaders of SpHtp1 and AVR3a are dimerisation sites, able to form heteromers. Furthermore, cell surface binding of both RxLR-proteins is mediated by an interaction with modified cell surface molecules. These results reveal a novel effector translocation route based on effector dimerisation and receptor modification, which could be highly relevant for a wide range of host-microbe interactions.

496. Fed-batch production of the hydrophobins RodA and RodB from *Aspergillus fumigatus* in host *Pichia pastoris.* M.H. Pedersen, I. Borodina, J.C. Frisvad and I. Søndergaard Systems Biology, Technical University of Denmark, Lyngby, Denmark. E-mail: mohp@bio.dtu.dk

Objectives: *Aspergillus fumigatus* expresses the hydrophobins RodA and RodB on the surface of its conidia. RodA is known to be important for the pathogenesis of the fungus, but the role of RodB is unknown. The aim was to produce recombinant RodA and RodB for further characterication. **Methods and materials**: The genes encoding hydrophobins RodA and RodB was amplified by RT-PCR from the total RNA isolated from *A. fumigatus* (AF296 strain), and cloned into expression vectors pPICZalphaA and pPICZB while adding a C-terminal 6xHis-tag. The linearized plasmids were transformed into *P. pastoris* strain X33. The expression of the RodA and RodB genes was first studied in culture flasks in buffered complex methanol medium as protein production was dependent on the methanol- induced AOX1 promoter. Later production was scaled up to a 2 L fed-batch fermentor. Hydrophobins were purified using His-select Nickel Affinity gel. The emulsifying properties of recombinant hydrophobins were investigated using oil-water emulsions studied by light microscopy. **Results**: Protein bands of expected size were detected by SDS-PAGE and western blotting in the fermentation broth. Fed-batch production yielded approximately 300 mg/l. Recombinant RodB showed good emulsifying properties. **Conclusion**: RodA and RodB from *A. fumigatus* were successfully produced by yeast host *Pichia pastoris* with good yields.

497. The role of Stp1, a secreted effector, in the biotrophic interaction of *Ustilago maydis* and its host plant maize. Liang Liang, Kerstin Schipper and Regine Kahmann Max Planck Institute for Terrestrial Microbiology, Dept. Organismic Interactions, Karl-von-Frisch-Strasse. 10, 35043 Marburg, Germany Email: liangl@mpi-marburg.mpg.de

After penetration of maize, the invading hyphae of the biotrophic smut fungus *Ustilago maydis* is surrounded by the plant plasma membrane which leads to the formation of an extended and tight interaction zone at the fungal/plant interface. For the establishment of a successful biotrophic interaction secreted effectors play crucial roles. One of these essential effectors is *stp1*, and orthologs of this gene are found only in smut fungi related to *U. maydis*. *stp1*mutants are non-pathogenic and arrest shortly after penetration. Deletion analysis revealed that the N- and C- terminal conserved regions of Stp1 are essential for protein function while the central glycine-rich domain is dispensable. In oomycetes, N-terminal RxLR motifs mediate effector uptake into plant cells. By generating mutations in all 6 degenerate RxLR-like motifs in the N-terminus of Stp1, we show that this domain is necessary for phospholipid binding but exclude that the RxLR-like motifs are required for Stp1 function. To elucidate whether Stp1 acts in the apoplast or is translocated to plant cells we have identified interactors by yeast two-hybrid screening using full length and truncated Stp1 as baits, respectively. 20 interactors from infected maize leaves were identified, coding for apoplastic as well as cytoplasmic plant proteins. Full length cDNA clones were isolated and their interaction with full length Stp1 was tested. For the most interesting interactors like rhamnogalacturonate lyase, the serine/threonine-protein kinase MHK, VIP2, CCR4-NOT transcription complex subunit and a cysteine protease 1, we are verifying the interaction with Stp1 by other in vitro and in vivo techniques and will present the latest results.

498. A fungal symbiont of plant-roots modulates mycotoxin gene expression in the pathogen *Fusarium sambucinum*. Youssef Ismail[1], Susan McCormick[2] and Mohamed Hijri[1]. [1] Université de Montréal, Département de sciences biologiques, Institut de recherche en biologie végétale (IRBV), 4101 rue Sherbrooke Est, Montreal, QC, H1X 2B2, Canada. [2] Bacterial Foodborne Pathogens and Mycology Research Unit, NCAUR, U.S. Department of Agriculture, 1815 N University St, Peoria, Illinois, 61604, USA.

Fusarium trichothecenes are fungal toxins that cause disease on infected plants and, more importantly, health problems for humans and animals that consume infected fruits or vegetables. Unfortunately, there are few methods for controlling the growth of mycotoxin production pathogens. In this study, we isolated and characterized sixteen *Fusarium* strains from naturally infected potato plants in the field. Pathogenicity tests were carried out in the greenhouse to evaluate the virulence of the trains on potato plants as well as their trichothecene production capacity and the most aggressive stain selected for further study. This strain, identified as *F. sambucinum*, was used to determine if trichothecene gene expression was affected by the symbiotic arbuscular mycorrhizal (AM) fungus *Glomus irregulare*. AM fungi form symbioses with plant roots, improving their mineral uptake and protecting plants against soil- borne pathogens. We find that G. irregulare significantly inhibits *F. sambucinum* growth. We also find, using RT- PCR assays to assess the relative expression of trichothecene genes, that in the presence of the AM fungus *G. irregulare, F. sambucinum* genes TRI5 and TRI6 were up-regulated, while TRI4, TRI13 and TRI101 were down regulated. We conclude that AM fungi can indeed modulate mycotoxin gene expression of a plant fungal pathogen. This previously undescribed effect may be a mechanism important for biological control and has fascinating implications for advancing our knowledge of plant-microbe interactions and controlling plant pathogens.

499. A novel, cysteine-rich fungal effector triggers light-dependent susceptibility in the wheat-*Stagonospora nodorum* interaction. Zhaohui Liu[1], Zengcui Zhang[1], Justin D. Faris[2], Richard P. Oliver[3], Peter S. Solomon[4], Timothy L. Friesen[1,2] [1]Department of Plant Pathology, North Dakota State University, Fargo, ND; [2] Northern Crop Science Lab, USDA-ARS, Fargo, ND; [3]Department of Environment & Agriculture, Curtin University, Perth, Australia; [4]School of Biology, The Australian National University, Canberra, Australia.

SnTox1 was the first necrotrophic effector identified in *S. nodorum*, and was shown to induce necrosis on wheat lines carrying *Snn1*. To isolate the SnTox1-encoding gene, we used bioinformatics tools followed by heterologous expression in *Pichia pastoris. SnTox1* encodes a 117 aa protein with the first 17 predicted as a signal peptide, and strikingly, the mature protein contains 16 cysteines. The transformation of *SnTox1* into an avirulent isolate was sufficient to make the strain pathogenic. Additionally, the deletion of *SnTox1* in virulent isolates renders the *SnTox1* mutant nonpathogenic on the *Snn1* differential line. The SnTox1-Snn1 interaction involves an oxidative burst, DNA laddering, and defense gene expression, all hallmarks of programmed cell death. In the absence of light, SnTox1-induced necrosis is blocked along with the disease development induced by the SnTox1-Snn1 interaction. By comparing the infection processes of a GFP-tagged avirulent isolate and the same isolate transformed with *SnTox1*, we conclude that SnTox1 plays a critical role in the initial penetration and subsequent proliferation in the host. This research provides important insights into the molecular basis of the wheat-*S. nodorum* interaction, a model for necrotrophic pathosystems.

500. Withdrawn

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

501. The *velvet* gene of *Fusarium graminearum* positively regulates virulence and trichothecenes biosynthesis. Christian Barreau[1], Jawad Merhej[1], Ludovic Saint-Felix[1], Martin Urban [2], Kim E. Hammond-Kosack[2] and Florence Forget-Richard[1]. [1]UR1264 MycSA INRA Bordeaux BP81 33883 Villenave d'Ornon cedex France. [2]Departement of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Herts, AL5 2JQ, U.K.

The filamentous fungus *Fusarium graminearum* infects cereals plants and causes "Fusarium Head Blight". During infection, it produces mycotoxins belonging to trichothecenes family which accumulate in the grains. Although the *Tri* genes implicated in the biosynthesis of trichothecene were identified, the global regulation of the toxin biosynthesis is still enigmatic. It has been recently demonstrated in *Aspergillus nidulans* that *veA* is the key component of the VelB/VeA/LaeA complex coordinating light signal with fungal development and secondary metabolism (Bayram et al. (2008)Science 320:1504-150). We identified *FgVe1*, the *velvet* gene from *F. graminearum* strain CBS 185.32 and disrupted this gene. Inactivation of *FgVe1* suppresses aerial hyphae formation and reduces the production of conidia. Complementation with the cloned *FgVe1* gene restores the wild type phenotype. Studies with these two mutants showed that *FgVe1* positively regulates the expression of *Tri* genes and production of trichothecenes. Inoculation on flowering wheat plants seedlings also showed that *FgVe1* is a positive regulator of virulence in *F. graminearum*.

502. Functional characterization of the *Fusarium virguliforme* NLP family. Sanchali Ghosh, Shannon Ritter and Sarah F. Covert Warnell School of Forestry and Natural Resources University of Georgia, Athens, GA 30602

Soybean sudden death syndrome (SDS) is a wide-spread disease caused by the fungus *Fusarium virguliforme*. Foliar necrosis, caused by *F. virguliforme*-secreted toxins, is a characteristic symptom of SDS. Because some Nep1-like proteins (NLPs), have been shown to cause necrosis and cell death in host plants, the goal of this project is to determine if *F. virguliforme*â€™s NLPs contribute to its pathogenicity on soybean. A phylogenetic analysis of NLPs in 4 sequenced *Fusarium* species identified 4 NLP subfamilies. Degenerate primers designed for each of the 4 subfamilies amplified 4 different NLP gene fragments from *F. virguliforme* genomic DNA. Complete coding sequences for each gene were collected via genome walking and fragments of each were inserted into the pTROYA RNAi silencing vector by Gateway cloning. Efforts to create knockdown mutants with these constructs are underway. Three additional NLPs were identified in the recently released draft genome sequence of *F. virguliforme*. Quantitative real-time PCR analysis to monitor expression levels of all 7 NLPs in *F. virguliforme*-infected soybean root samples are also underway.

503. Pathogen-caused release of linolenic acid suppresses plant defense by inhibition of callose synthesis in wheat. Voigt, Christian [1], Goebel, Cornelia [2], Bode, Rainer [2], Feussner, Ivo [2], and Schaefer, Wilhelm [1]. [1] Molecular Phytopathology, Biocenter Klein Flottbek, University of Hamburg, Germany. [2] Department of Plant Biochemistry, Georg August University, Goettingen, Germany.

The precise function of callose in papillae has not been shown unequivocally. We demonstrate that upon infection of wheat spikes with the fungal plant pathogen *Fusarium graminearum*, callose synthase activity and callose deposition are suppressed, and wheat is susceptible to fungal spreading. The secreted lipase FGL1 is an important virulence factor for *F. graminearum*. In contrast to *F. graminearum* wild-type, the lipase-deficient Î”fgl1 mutant is unable to suppress wheat callose synthesis. Wheat spikes are resistant to colonization by this mutant. Long- chain unsaturated free fatty acids (FFA) inhibit plant callose synthesis in vitro and in planta; and the previously observed resistance of the wheat spike to Î”fgl1 is broken. The lipase-deficient fungal mutant is able to colonize the spike. Analysis of the FFA level in wheat spikes during infection revealed an elevated linolenic acid concentration during *F. graminearum* wild-type compared to Î”fgl1 infection. We conclude that linolenic acid plays a decisive role in callose synthesis suppression during wheat â€“ *F. graminearum* interaction. A proposed model explains this novel mechanism of plant defense suppression by pathogen-caused increase in FFA due to lipase secretion.

504. Nutrient regulation of parasitism genes in the nematophagous fungus *Pochonia chlamydosporia*. Elaine Ward, Brian Kerry, Rosa Manzanilla-López, and Penny Hirsch. Plant Pathology and Microbiology Dept., Rothamsted Research, Harpenden, Herts. AL5 2JQ, UK. Elaine.ward@bbsrc.ac.uk .

The rhizosphere-inhabiting ascomycete fungus *Pochonia chlamydosporia* is used as a biological control agent against plant parasitic nematodes. It grows saprophytically on the surface of plant roots and switches to become a parasite on contact with nematode egg masses and cysts. We have identified fungal genes involved in the early stages of egg infection and are investigating how their expression is affected by nutritional factors. Molecular variation was studied in one of these genes, VCP1, an alkaline serine protease. The sequences of the upstream flanking regions of this gene were highly conserved and contained putative regulatory motifs. Expression of the VCP1 gene was investigated in media containing different nutrients using colorimetric assays of enzyme activity and quantitative real-time RT-PCR. A better understanding of nutrient regulation of parasitism genes is important for optimising the exploitation of *P. chlamydosporia*. In particular we need to understand what effects commonly-used soil amendments may have on its efficacy as a biological control agent. Rothamsted Research receives grant aided support from the Biotechnology and Biological Sciences Research Council of the UK. This work is supported by funding from BBSRC and DFID (Grant BB/F003994/1).

505. The impact of the mycorrhizal symbiosis on the transcriptome of *Laccaria bicolor* and Poplar. Annegret Kohler[1], Jonathan M. Plett[1], Emilie Tisserant[1], Minna Kemppainen[2], Valérie Legué[1], Claire Veneault-Fourrey[1], Annick Brun[1], Alejandro G. Pardo[2] and Francis Martin[1] [1] UMR INRA-UHP 1136 Interactions Arbres/Micro-organismes, Centre INRA de Nancy, 54280 Champenoux, France. [2] Laboratorio de Micología Molecular, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes and CONICET. Roque Sáenz Peña 352, B1876 Bernal, Provincia de Buenos Aires, Argentina.

Mycorrhizal symbioses are universal in terrestrial ecosystems and may have been fundamental to land colonization by plants. Boreal and temperate forests all depend on ectomycorrhizae. These fungi are not an evolutionarily distinct group, but rather evolved several times from saprotrophic ancestors. Identification of the primary factors that regulate symbiotic development and metabolic activity will therefore open the door to understand the role of ectomycorrhizae in plant development and physiology, allowing the full ecological significance of this symbiosis to be explored. With the genome sequences of *Populus trichocarpa* and *Laccaria bicolor* in hand, we were able to investigate the transcriptome of both partners during mycorrhizal development. By using Nimblegen whole genome expression arrays and RNA-Seq we identified candidate genes from the fungus and its host tree. Amongst the most highly symbiosis up- regulated transcripts in *Laccaria bicolor* were several small secreted proteins. From amidst these small secreted proteins we have demonstrated that *MYCORRHIZAL iNDUCED SMALL SECRETED PROTEIN7* is an indispensable signal needed for the establishment of symbiosis. MiSSP7 is secreted by the fungus upon receipt of diffusible signals from plant roots, imported into the plant cell via endocytosis and targeted to the plant nucleus where it alters the transcriptomic fate of the plant cell. Further, *L. bicolor* transformants with severely reduced expression of MiSSP7 do not form functional mycorrhia with poplar roots. Martin, F. et al., The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature 452, 88-92 (2008)

506. Conservation of dimorphism and virulence in a non-canonical dimorphic fungus: *Cryptococcus neoformans.* Xiaorong Lin, Texas A&M University, College Station, TX, xlin@mail.bio.tamu.edu

A prominent feature shared by the majority of human pathogenic fungi is dimorphism. The ability to switch between the yeast and the filamentous form is tightly associated with virulence. For endogenously acquired pathogens, such as *Candida albicans*, the commensal state is in the yeast form while the parasitical state is associated with the filamentous form. In contrast, for other ascomyceteous dimorphic fungi that are acquired from the environment, the filamentous form is saprophytic and the yeast form is considered parasitic. Thus, knowledge about the molecular mechanism underlying the association of dimorphism and virulence will help to understand the observed divergence of morphotypes in virulence observed in these two types of dimorphic fungi. We recently discovered that the transcription factor Znf2 quantitatively controls hyphal morphogenesis in Cryptococcus neoformans and negatively governs virulence. This basidiomycetous yeast is not typically considered dimorphic due to the rareness of filamentation events and the intimate association between filamentation and mating. However, our characterization of Znf2 and its targets demonstrate that this pathway controls the remodeling of cell surface, which represents the interface between fungal cells and the host, and ultimately determines morphotype. Thus further investigation of Znf2 and its targets will provide critical insights into the general mechanism underlying dimorphism and virulence among evolutionarily diverse fungal species.

507. ChLaeA and ChVeA regulate T-toxin production, oxidative stress responses, and reproductive and pathogenic development of *Cochliobolus heterostrophus*. Dongliang Wu, Ning Zhang, May Yee Choi and B. Gillian Turgeon. Dept. of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY. dw358@cornell.edu

Race T of the maize pathogen, *Cochliobolus heterostrophus*, produces T-toxin, a secondary metabolite promoting high virulence to maize carrying Texas male sterile cytoplasm, and also other secondary metabolites, such as siderophores, centrally involved in managing iron metabolism, oxidative stress, and virulence. In Aspergillus, VeA and LaeA, two velvet complex components, coordinate secondary metabolism and differentiation in response to light signals. We report that expression of nine genes known to be required for T-toxin production are upregulated in the dark in WT; in *laeA* and *veA* mutants, expression is much reduced and light regulation is erased, making these the first factors known to regulate T-toxin production. Because *ChlaeA* and *ChveA* mutants are also altered in responses to oxidative stress, expression of genes involved in oxidative stress management were examined. Expression signatures of *NPS6* (extracellular siderophore) and *SSK1* and *SKN7* (histidine kinase response regulators), were unaltered in the mutants, while expression of *CAT3* (catalase) was reduced. ChLaeA and ChVeA inhibit expression of melanin pathway genes in WT. *laeA* and *veA* mutants are altered in reproductive abilities showing increased asexual sporulation and abnormal sexual differentiation (female sterile). Virulence of the mutants was altered on both T- and normal cytoplasm maize, indicating that both basic pathogenicity and high virulence due to T-toxin were affected. Thus, ChLaeA and ChVeA positively regulate T-toxin biosynthesis, oxidative stress responses, virulence, sexual development, and aerial hyphal growth, and negatively control melanin biosynthesis and asexual differentiation. This is the first report that ChLaeA and ChVeA play roles in oxidative stress responses.

508. *Aspergillus fumigatus* conidia modulate the endocytic pathway of alveolar macrophages. Andreas Thywissen[1, 3], Thorsten Heinekamp[1], Hans-Martin Dahse[2], Peter F. Zipfel[2, 3], and Axel A. Brakhage[1, 3]. [1] Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute (HKI), Department of Molecular and Applied Microbiology, Jena, Germany. [2] Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute (HKI), Department of Infection Biology, Jena, Germany. [3] Friedrich Schiller University, Jena, Germany.

The mould *Aspergillus fumigatus* is the main causative agent of invasive pulmonary aspergillosis in immunocompromised patients. Infection starts with the inhalation of *A. fumigatus* conidia that germinate in the lung. Professional phagocytes like alveolar macrophages contribute to the efficient clearance of fungi from the lung by phagocytosis and degradation of conidia followed by release of chemokines and cytokines in order to trigger neutrophil migration at the site of infection. In the immunocompromized host, at least some conidia are able to evade macrophage degradation, resulting in germination and outgrowth of intracellularly residing spores. Therefore, conidia must be able to evade recognition and processing by phagocytes. The avirulent *pksP* mutant of *A. fumigatus* lacking the melanin layer present on wild-type conidia exhibited increased phagocytosis by macrophages apparently due to the loss of masking immunogenic glucan-structures. Furthermore, by analysing phagolysosome fusion and acidification we show that intracellular processing of *pksP* mutant conidia is drastically increased in comparison to wild-type conidia, suggesting that *A. fumigatus* conidia interfere with the endocytosis pathway, similar to obligate human pathogens like *Legionella sp*. or *Mycobacterium sp*.. The process by which wild-type conidia mediate endocytotic alterations seems to be connected to the surface structure of melanized conidia but is independent of the presence of a functional RodA-derived rodlet layer. Moreover, inhibition of phagolysosome acidification by macrophages is controlled by the fungal cAMP signaling pathway.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

509. Adhesion of *Ustilago maydis* filaments at the onset of pathogenic development. Kai Hofmann and Joerg Kaemper. Karlsruhe Institute of Technology, IAB Dept. of Genetics, D-76187 Karlsruhe, Germany. e-mail: Kai.Hofmann@kit.edu .

In pathogenic fungi, adhesion to surfaces is considered an important event in disease establishment. Adhesion of the phytopathogenic basidiomycete *Ustilago maydis* is tightly linked to the formation of filaments on the plant surface that follows the establishment of the infectious dikaryon. Filamentation of the fungus can also be induced solely by contact with a given hydrophobic surface like Polytetrafluoroethylene. The factors propagating hyphal adhesion in *U. maydis* are still largely unknown, and identification of distinct factors by comparative sequence analyses is hindered by the heterogeneity of involved gene products and their functional redundancies. In an attempt to circumvent these difficulties we adapted a method developed for identification of *Candida albicans* adhesion-promoting genes. We use a parallel plate shear flow assay to screen a *U. maydis* cDNA library expressed in adhesion-deficient *Saccharomyces cerevisiae* cells to identify genes that enhance adhesive capabilities of yeast cells to hydrophobic surfaces. The use of a parallel plate flow chamber provides quantitative reproducible measurements of cell detachment from these surfaces by applying a known shear stress under conditions of laminar flow. Identified genes and respective gene products will subsequently be analyzed and characterized in *U. maydis*. We expect that this approach will lead to the identification of novel adhesins and regulatory elements controlling surface adhesion.

510. Feasibility studies for the development of VHH-based detection systems. Lämmel, Jana[1], Matthias Brock [2] and Uwe Horn [1 1] Bio Pilot Plant, Leibniz-Institute for Natural Product Research and Infection Biology e. V. (Hans-Knöll- Institute), Jena, Germany [2] JRG Microbial Biochemistry and Physiology, Leibniz-Institute for Natural Product Research and Infection Biology e. V. (Hans-Knöll-Institute), Jena, Germany

Since fungal pathogens that cause invasive infections show resistance to different therapeutical drugs hence the treatment is difficult. A fast und precise identification of the etiological agent is essential for successful therapy. Among established methods like cultivation on selective media, microscopy and histology, the popularity of molecular methods for detection is rapidly increasing. Serological test systems are either based on the detection of antibody response or the detection of specific antigens of the pathogen. The use of monoclonal antibodies of immunized mammals is the method of choice for the latter. As an alternative, the employment of fully synthetic antibody libraries based on camelide heavy-chain antibodies becomes more interesting. These antibodies lack the light chain and the variable domain (VHH) is fused to the gIII-Protein of M13-phages. The libraries are then read out by phage display. The frame work of our library enables easy fusion of enriched and specific VHH domains to alkaline phosphatase. This leads to dimerization thus increasing sensitivity and facilitates detection of the antibody. Due to their smaller size, VHH domains show higher stability and increased capability of binding to epitopes which are rather inaccessible for conventional IgGs.

511. Metabolomic priming by a secreted fungal effector. Armin Djamei, Kerstin Schipper, Franziska Rabe, Regine Kahmann Max Planck Institut für terrestrische Mikrobiologie Karl-von-Frisch-Straße D-35043 Marburg / Germany

A successful colonization of plants by pathogens requires active effector-mediated suppression of defense responses. Here we show that the biotrophic fungus *Ustilago maydis* secretes an enzymatically active chorismate mutase, Cmu1. This enzyme is taken up locally by infected plant cells and then spreads to neighboring cells. Nonregulated enzymatic activity of the fungal chorismate mutase and interactions with cytoplasmic plant chorismate mutases are likely to be responsible for a re-channeling of the shikimate pathway. The comparison of the metabolomes of maize plants infected either with *cmu1*-deletion mutant or its progenitor strain showed significant changes in phenylpropanoid pathway derivates and phytohormone levels. Based on these findings, we propose a model in which the virulence factor Cmu1 actively reduces salicylic acid levels, thereby allowing the suppression of PAMP-triggered defense responses. Through this “metabolic priming”, the maize plant is prepared for a successful infection by *U. maydis*. Our study describes a novel strategy for host modulation that might be used by a wide range of biotrophic plant pathogens.

512. The role of a signal peptidase component in the pathogenicity of *Colletotrichum graminicola* to maize. Ester Buiate[1]; Lisa Vaillancourt[1*] *vaillan@uky.edu, 1Department of Plant Pathology, University of Kentucky 201F Plant Science Building 1405 Veterans Driver Lexington-KY 40546-0312

Anthracnose stalk rot, caused by *Colletotrichum graminicola*, is one of the most damaging diseases of maize. An insertional mutation in the 3’UTR of the CPR1 gene, encoding a non-catalytic component of the ER localized signal peptidase, was produced by REMI mutagenesis. The mutant is normal except for a slightly reduced growth rate *in vitro*, but it is non-pathogenic to maize leaves and stalks, failing to establish a successful biotrophic infection. The plant is known to respond to fungal attack by production of ROS and induction of oxidative stress, and fungi are presumed to experience nutritional and other stresses. Unfortunately we know very little at present about the types of stresses that pathogenic fungi actually encounter *in planta*. Our objective is use a fluorescent reporter system to identify stresses that occur during biotrophic development of *C. graminicola in planta*, and to test the hypothesis that CPR1 has a specific role in adaptation to these stresses. The CPR1 mutant is more sensitive to ER stress induced by chemicals, to oxidative stress and to heat stress *in vitro*, but not to nitrogen starvation. Experiments are continuing to further characterize stress responses of the mutant *in vitro* and *in planta*.

513. Nitric Oxide Metabolism and the Role of NO Detoxifying Flavohaemoglobin in *Fusarium verticillioides*. Thomas Baldwin[1], Anthony Glenn[2] [1]Plant Pathology, University of Georgia, Athens Georgia. [2] USDA, ARS, Russell Research Center, Toxicology & Mycotoxin Research Unit, Athens, Georgia.

Nitric oxide (NO) is a small free radical, highly reactive, and responsible for both cytotoxic and cytostimulant effects in the cell. The dual nature of NO makes it the perfect candidate for both molecular signaling and an effective first line of defense against pathogens. *Fusarium verticillioides* is a non-obligate pathogen causing a number of maize diseases. One possible determinate of its pathogenesis could be the regulation of NO crosstalk with its maize host. Detoxification of NO is a known pathogenicity factor for the fungal human pathogen *Candida albicans* and the bacterial plant pathogen *Erwinia chrysanthemi*, requiring a flavohaemoglobin protein for this detoxification (CaYHB1 and HmpX, respectively). BLASTP search of the *F. verticillioides* genome revealed two putative flavohaemoglobin homologs, denoted *NOD1* and *NOD2* (for Nitric Oxide Dioxygenase). Δ*NOD1* mutants sequestered NO in vacuoles, whereas wild type accumulated NO in the cytoplasm. This accumulation may be a cellular response to the inability to detoxify the NO generated by the fungus. Δ*NOD2* mutants are being developed and both gene deletions will be evaluated for growth and development phenotypes and their virulence against maize seedlings.

514. Deconvoluting the *Neotyphodium coenophialum* genome. Carolyn Young[1], Ranamalie Amarasinghe[1], Johanna Takach[1], Patrick Zhao[1], Jennifer S. Webb[2], Neil Moore[2], Jolanta Jaromczyk[2], Charles T. Bullock[2], Jerzy W. Jaromczyk[2], Christopher L. Schardl[2]. [1]The Samuel Roberts Noble Foundation, Ardmore, Oklahoma; [2]University of Kentucky, Lexington Kentucky.

The epichloë endophyte *Neotyphodium coenophialum* forms a mutualistic association with the cool-season grass, tall fescue (*Lolium arundinaceum*). The wide range of benefits the endophyte provides its host has made tall fescue an agriculturally important grass. Unfortunately, some isolates are known to cause toxicity to grazing livestock due to the production of ergot alkaloids. To unravel the complexity of the endophyte-grass association, we have embarked on sequencing the *N. coenophialum* genome and identifying symbiosis induced genes. *N. coenophialum* is considered an interspecific hybrid consisting of origins from *E. festucae*, *E. typhina* and a *Lolium*- associated endophyte closely related to *E. baconii*, with an estimated genome size of 57Mb. We have started genome sequencing using cosmid/fosmid end sequencing and '454' shot-gun and paired end reads. Initial assembly of the 454 generated data indicated a larger genome size (95 Mb) than originally estimated. Transcriptome comparison using Illumina sequencing of mRNA from in planta vs in culture conditions showed some of the most highly expressed genes were those required for production of lolines and ergot alkaloids. Laser capture microscopy will be used to dissect the endophyte from the host to enrich for endophyte transcripts in planta and will be compared with the Illumina data. Deconvolution of the *N. coenophialum* genome will provide insight into the impact of repetitive elements in genome evolution and allow us to see what gene families, including those of secondary metabolism, have been retained or lost during hybridization.

515. *FGP1* is involved in pathogenicity and hyphal morphological changes during toxin production in *Fusarium graminearum*. Wilfried Jonkers[1], Karen Hilburn[1], Yanhong Dong[2] and H. Corby Kistler[1,2]. [1]Cereal Disease Lab, ARS-USDA, 1551 Lindig street, St. Paul Mn 55108 USA. [2]Department of Plant pathology, University of Minnesota, St. Paul Mn 55108 USA. Email: Jonke007@umn.edu

FGP1 (***F****. graminearum* ***G****TI1/****P****AC2* ***1***) is, like its counterpart *SGE1* from *Fusarium oxysporum*, required for pathogenicity and normal conidiation. Normally, the fungus is able to spread through the entire wheat head when one spikelet is inoculated, but no spread is observed when wheat spikelets are inoculation with the delta*fgp1* mutant. During the first stages of infection, the wild type strain PH-1 produces trichothecenes, toxins required for pathogen and symptom spread. No toxins are detected with the delta*fgp1* strain, neither *in planta* nor in toxin induction medium containing putrescine. We found that during toxin production *in vitro*, a morphological change is observed in the wild type; hyphal tips swell and bulbous subapical structures containing large, vacuolar cell compartments are formed. We hypothesize these bulbous structures may facilitate toxin production. In the mutant these morphological changes are observed to a much lower extent and the largest bulbous forms are absent, suggesting that the incomplete formation of these structures might be the cause of the absence of toxin production. By comparing the whole genome expression profiles in WT and the delta*fgp1* mutant during this transition, we hope to discover the transcriptional and underlying genetic basis for this morphological change during toxin production.

516. Characterization of the fungicidal activity of sertraline. Bing Zhai and Xiaorong Lin Room 435, Biological Sciences Building West 3258 TAMU College Station, TX 77843-3258 Email: bzhai@mail.bio.tamu.edu

Cryptococcus neoformans is the etiologic agent of cryptococcal meningitis that causes more than half a million death each year. However, except some fungistatic azole antifungals (e.g. fluconazole), there is no drug that can penetrate brain for the treatment of fungal meningitis. We have recently discovered that the antidepressant drug sertraline possesses a potent fungicidal activity against all fungal species tested *in vitro*. It was especially effective against *Cryptococcus*, including fluconazole resistant isolates, at clinical relevant concentrations. These results indicate a potential application of sertraline in treating cryptococcal meningitis, particularly given the neurotropic nature of this drug. Together with studies from other groups, our study reveals that sertraline has a wide spectrum antiproliferative activity against virus, bacteria, fungi, and mammalian tumor cells. In order to identify the fungal target of sertraline, the *Saccharomyces* whole genome deletion set was screened. A group of mutants defective in translation process were found to display altered sensitivity towards sertraline. *In vitro* translation assay with yeast cell extract using luciferase as a report protein showed that the luciferase activity was inhibited at the presence of sertraline. Our results suggest that the translation process could serve as a possible target and sertraline (or its analogs) holds a promise as an antifungal that can penetrate the brain to treat fungal meningitis.

517. Identifying regulators of virulence traits in the fungal pathogen *H. capsulatum*. Sarah A. Gilmore and Anita Sil; University of California, San Francisco, San Francisco, CA.

Histoplasma capsulatum (Hc) is a human fungal pathogen that is a leading cause of both endemic and opportunistic fungal infections worldwide. One characteristic of Hc that is thought to contribute to its success as a pathogen is its ability to exist in two distinct, temperature-dependent morphological states. In the soil Hc grows as filaments, but transitions to a yeast form that causes disease, disease-causing form during growth in a mammalian host (37˚C). Notably, over 700 temperature-regulated transcripts are more highly expressed in yeast cells than Hc filaments, but it is unclear which of these genes affect pathogenesis in the host. To begin to understand how the network of temperature-induced yeast-phase genes contributes to Hc pathogenesis, I have identified four candidate transcription factors (Rpn4, Csr1, Cph1, and Nrg1) that are enriched in the yeast versus filamentous phase of Hc growth. Morphological examination of RNAi knockdown strains generated for each transcription factor revealed that RPN4, CSR1, and NRG1 RNAi strains grew in the yeast form at 37˚C; CPH1, conversely, grew as a mixture of filaments and yeast cells at 37˚C. This suggests that Cph1 may play a role in controlling yeast cell morphology, while Csr1, Nrg1, and Rpn4 regulate functions independent of yeast phase morphology. Work is ongoing to identify the regulons of each transcription factor via gene expression profiling. Ultimately, this work will allow the systematic identification of gene modules that are of interest to Hc biology.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

518. *Pyrenophora tritici-repentis*: Defining factors required for pathogenicity. Wade H. Holman, Lynda M. Ciuffetti Department of Botany and Plant Pathology, Oregon State University.

Pyrenophora tritici-repentis (Ptr) is a pathogenic fungus and the causal agent of tan spot of wheat (Triticum aestivum). Ptr ToxA (ToxA) is a proteinaceous host-selective toxin produced by Ptr that induces necrosis (cell death) in toxin- sensitive wheat cultivars. The ToxA mode-of-action involves internalization into sensitive wheat mesophyll cells, localization to the chloroplast, and accumulation of reactive oxygen species. However, neither the mechanism by which ToxA targets the chloroplast nor all of the ToxA-interacting partners in wheat are known. We have shown that internal expression of ToxA in tobacco (Nicotiana benthamiana) is sufficient to induce cell death. Therefore, tobacco provides an ideal model system to define proteins important for ToxA sensitivity. By silencing genes in tobacco and monitoring the loss of ToxA-induced cell death we can identify those proteins that contribute to the development of necrosis. A total of 1,152 genes have been silenced and 88 candidate genes identified that affect ToxA-induced cell death. A secondary screen will be used to validate these results. The putative function of the silenced genes that contribute to ToxA sensitivity will be deduced based on sequence similarities to genes of known function. Where possible, ToxA-induced transcriptome changes in wheat will be used to compliment these findings. The results of this study will provide further insight toward understanding the molecular interactions between ToxA and wheat proteins.

519. Mechanisms of *Candida albicans* invasion of Brain Endothelial Cells Revealed by Investigation of *C. albicans* Vps51. Yaoping Liu [1], Rahul Mittal [2], Norma Solis [1], Quynh T. Phan [1], Nemani V. Prasadarao [2], Scott G. Filler [1, 3]. [1] Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA. [2] Childrens Hospital Los Angeles, Los Angeles, CA. [3] David Geffen School of Medicine at UCLA, Los Angeles, CA

In *Saccharomyces cerevisiae*, Vps51p forms a complex with Vps52p-54p and mediates protein sorting to the golgi. To investigate the function of this complex in *Candida albicans*, we constructed *vps51*Δ/Δ and *vps53*Δ/Δ mutant strains and tested their virulence in the murine model of disseminated candidiasis. Compared to mice infected with the wild-type strain, mice infected with either the *vps51*Δ/Δ or *vps53*Δ/Δ mutant had significantly fewer organisms in their kidneys and livers. Interestingly, mice infected with either mutant had a 70-fold higher brain fungal burden, suggesting that deletion of *VPS51* or *VPS53* results in enhanced trafficking to the brain. Consistent with this hypothesis, we found that the *vps51*Δ/Δ mutant had significantly increased capacity to invade human brain microvascular endothelial cells (HBMECs; 39% increase compared to wild-type) *in vitro*; but significantly impaired capacity to invade human umbilical vein endothelial cells (HUVECs; 55% reduction compared to wild-type). The heat shock protein Gp96 is expressed on the surface of HBMECs, but not HUVECs. Knockdown of gp96 by siRNA reduced HBMEC invasion by both wild-type *C. albicans* and the *vps51*Δ/Δ mutant. Overexpression of gp96 in HBMEC and CHO cells increased invasion by both *C. albicans* strains. Additional studies demonstrated that the *C. albicans* invasin, Als3p is overexpressed on the surface of the *vps51*Δ/Δ mutant, and this overexpression contributes to the enhance capacity of this mutant to invade HBMECs *in vitro* and traffic to the brain in mice. Thus, *C. albicans* Als3p binds to gp96 and mediates invasion of brain endothelial cells *in vitro* and *in vivo*.

520. Insight into transcriptional regulatory mechanisms controlling filamentation in *Candida albicans* under hypoxia. Adnane SELLAM, André NANTEL, Faiza TEBBJI, Christopher ASKEW, Malcolm WHITEWAY. McGill University, Montreal, PQ, Canada.

To gain insight into regulatory mechanisms controlling hyphae formation in response to low oxygen concentration in the opportunistic yeast *C. albicans* a compilation of mutants from various publicly available libraries were screened (648 mutant strains). In this work we focused our investigation on mutants of genes encoding for transcription factors, components of chromatin remodeling and histone modification complexes, and protein acting in different signaling pathways. The ability of mutants to form hyphae specifically under hypoxic condition was assessed in solid medium by scoring the filamentation of colonies peripheral regions. Filamentation screen identified 40 mutants with substantial morphology defect. We focused our investigation on the transcription factor Ahp1p whose mutant displayed a hyperfilamentation specifically under hypoxia. Transcriptional regulatory network associated with this factor was characterized using both expression profiling and ChIP-chip. The obtained results demonstrated that this factor is required to activate genes involved in iron uptake and many adhesin-encoding genes specifically under hypoxia. Transcriptional regulatory circuits implicating other regulators will be also presented and their role in the adaptation to hypoxia and filamentation will be discussed.

521. Sfp-Type 4'-phosphopantetheinyl transferase (CsPPT1) is required for lysine synthesis, tolerance to oxidative stress, and virulence in the plant pathogenic fungus *Cochliobolus sativus*. Yueqiang Leng and Shaobin Zhong*. Department of Plant Pathology, North Dakota State University, Fargo, ND 58108. *corresponding author, email: shaobin.zhong@ndsu.edu .

Polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) are the major enzymes involved in biosynthesis of secondary metabolites such as polyketides (PKs) and nonribosomal peptides (NRPs) in many filamentous fungi. However, all of the PKSs and NRPSs require 4'-phosphopantetheinylation at the conserved Ser residues for activation catalyzed by an enzyme called 4'-phosphopantetheinyl transferase (PPTase). PPTase not only activates PKSs and NRPSa but also is involved in primary metabolism (a-aminoadipate reductase [AAR]). In the genome sequence of the cereal fungal pathogen *Cochliobolus sativus*, we identified a gene (*CsPPT1*) homologous to the PPTase- encoding gene found in other fungal pathogens and generated PPTase deficient mutants (Δ*CsPPT1*). The Δ*CsPPT1* strains were auxotrophic for lysine, unable to synthesize melanin and hypersensitive to oxidative stress. Also, conidial productivity of the Δ*CsPPT1* strains was significantly reduced compared to the wild type strain. The Δ*CsPPT1* strains were able to penetrate barley leaves, but failed to cause the severe spot blotch disease symptom produced by the wild type strain on the barley cultivar Bowman. No significant difference in virulence was observed between the lysine-auxotrophic mutants (Δ*CsAAR*) and the wild type strain when inoculated on wounded barley leaves or when lysine was supplemented, indicating that *CsAAR* is not required by the fungus for virulence on barley. We hypothesize that an unknown virulence factor, presumably synthesized by PKSs or NRPSs in *C. sativus*, is directly responsible for high virulence on barley and the Sfp-type 4-PPTase encoded by *CsPPT1* is involved in activation of the enzyme(s) for synthesis of the virulence factor.

522. High affinity iron acquisition in virulence of the maize pathogen *Cochliobolus heterostrophus*. Bradford Condon, B. Gillian Turgeon. Cornell University, Ithaca, NY.

Iron is an essential micronutrient for nearly every organism on earth, including fungal plant pathogens. Plant host iron is bound to ferritin or other chelators, so successful pathogens must possess a method for high affinity iron acquisition to liberate it. Fungi have two known mechanisms for this. Many produce small Fe^{3+} chelating peptides called siderophores, which can be involved in iron acquisition or homeostasis. Fungi can also reduce Fe^{3+} at the cell membrane and transport it with a high affinity iron permease (Ftr1p), a process called reductive iron assimilation (RIA). The maize pathogen Cochliobolus heterostrophus possesses both RIA and two types of siderophore: intracellular siderophores for iron homeostasis produced by the nonribosomal peptide synthetase Nps2p, and extracellular siderophores produced by Nps6p. *nps6* and *nps2nps6* mutants display reduced virulence and increased sensitivity to low iron and oxidative stress. To test whether loss of RIA also affects virulence and sensitivity to low iron and oxidative stress, an ftr1-deletion strain was generated. Unlike *nps6*-deletion strains, *ftr1* strains are like WT with respect to these characteristics. *nps6ftr1* and *nps2nps6ftr1* triple mutants, generated by crossing *nps2/nps6* and *ftr1* mutants, however, display growth and asexual spore production defects, increased sensitivity to oxidative stress, and reduced virulence compared to *nps6* mutants. *nps2nps6ftr1* requires micromolar concentrations of supplemental iron for survival, and is capable of germinating on the host, but not penetrating. High affinity iron acquisition mechanistic preferences and corresponding impact on virulence of the maize necrotroph, *C. heterostrophus*, suggest a role for siderophores in virulence beyond nutrition.

523. GFP-expressing *Metarhizium* spp. varying in virulence demonstrate differential development on diverse arthropod hosts. Dana Ment[1,2], Galina Gindin[2], Alice C.L. Churchill[3], Asael Rot[4], Bruno G.G. Donzelli[3], Eduard Belausov[2], Itamar Glazer[2] and Michael Samish[2]. [1] Robert H. Smith Faculty of Agriculture, Food and Environment The Hebrew University of Jerusalem, Rehovot 76100 Israel; [2] Entomology and the Nematology and Chemistry units, Institute of Plant Protection, ARO, The Volcani Center, P.O.B. 6, Bet-Dagan, 50250 Israel; [3] Department of Plant Pathology and Plant-Microbe Biology, Tower Road, Cornell University, Ithaca, NY 14853 USA; and [4] The Kimron Veterinary Institute, P.O.B. 12, Bet-Dagan, 50250 Israel, e-mail: danam@volcani.agri.gov.il

Fungi in the *Metarhizium anisopliae* complex (Hypocreales: Clavicipitaceae) include both broad and narrow host- range pathogens of arthropods. An understanding of the early interactions between pathogens and hosts that support or limit infections is necessary for efficacious deployment of entomopathogens as biological control agents of pest arthropods. We and others hypothesized that the composition of tick cuticles is a major factor determining host susceptibility to fungal infection. In *in vitro* studies, we demonstrated previously that cuticular compounds from resistant and susceptible ticks differentially modulate fungus development, specifically conidium germination and appressorium differentiation. In the current study, observations of GFP-expressing *Metarhizium* spp. that vary in virulence on tick and lepidopteran hosts demonstrated that the surface of a resistant host may support conidial germination and hyphal growth but, in contrast with growth on susceptible hosts, restricts penetration of hyphae into the hemocoel. Furthermore, cuticle from a resistant tick host appears to contain compounds that actively suppress fungal growth and survival on the tick surface, as visualized by loss of GFP expression and propidium iodide staining of hyphae. We are applying integrated, multidisciplinary approaches to test the hypothesis that tick host cuticular components modulate fungal development *in vivo*. These include analyses of soluble cuticular proteins, epicuticular lipids and tick pheromones effecting fungal differentiation. Additionally, expression of fungal cuticle-degrading enzymes in response to cuticle from different hosts is being evaluated. Understanding the differential induction of *Metarhizium* spp. virulence factors by distinct host cuticular compounds will enable better utilization of the fungus in pest control.

524. Identification of *Verticillium* virulence factors using proteomics Koste A. Yadeta. Peter van Esse, Patrick Wijten, Ronnie de Jonge, and Bart P.H.J. Thomma Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands. Koste.Yadeta@wur.nl

The soil-borne fungus *Verticillium dahliae* is one of the most notorious plant pathogens that causes vascular wilt diseases on over 200 dicotelydenous plant species. Despite its economic importance, little is known about the mechanisms that enable this fungus to infect such a wide array of host plants. It is generally accepted that the *in planta* secreted pathogen proteins (secretome) contains effectors that determine pathogenicity. To identify *V. dahliae* pathogenicity determinants, we performed proteomic analyses on *V. dahliae* grown *in vitro* in the presence and absence of tomato xylem fluid. In total 404 proteins were detected in *Verticillium* cultures without xylem fluid whereas the number of proteins detected in the presence of xylem fluid was increased to 489. We also analyzed the secretome of *V. dahliae* grown solely on tomato xylem fluid and identified 392 proteins. The *V. dahliae* genome is predicted to encode 780 secretome proteins. Only about 37 % of the proteins detected in the MS analyses contain a signal peptide for secretion. These proteins potentially determine the outcome of *Verticillium*- host interaction and are candidates for functional analysis.

525. Understanding how legumes distinguish friends and foes by using the *Medicago truncatula*/*Aphanomyces euteiches* pathosystem. Bernard Dumas, Amaury Nars, Diana Ramirez, Thomas Rey, Claude Lafitte, Arnaud Bottin, Elodie Gaulin,Christophe Jacquet UMR 5546 CNRS-Université Paul Sabatier, Castanet-Tolosan France, dumas@scsv.ups-tlse.fr

Symbiotic bacterial signals, such as the lipochitoolisaccharides Nod factors, are essential for the establishment of legume root symbiosis. Related fungal signals, such as chitin fragments, induce defense reactions, raising the question of how roots distinguish friends and foes. To address this issue, we have focussed our work on the interaction between *Medicago truncatula* and the oomycete root pathogen *Aphanomyces euteiches*. Biochemical analyses of *A. euteiches* cell wall revealed the presence of high levels of N-acetyl glucosamine, and chitin-like materials are now characterized to understand how the host distinguishes *A. euteiches* chitosaccharide-derived fragments from Nod factors. It is now known that microbial effectors interfere with the perception of elicitors. Mining of an *A. euteiches* ESTs database revealed the presence of putative translocated effectors belonging to the Crinkle family of oomycete effectors but not to the RXLR family. Transgenic *M. truncatula* roots expressing these genes are being produced and effects on the responses towards pathogenic and symbiotic signals will be evaluated. Together, our results show that the *Medicago*- Aphanomyces interaction provides is a valuable system to decipher the complex signalling network which establishes between plants and symbiotic or pathogenic microorganisms.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

526. Comparative secretome analysis of Verticillium albo-atrum isolates from hop. Stanislav Mandelc[1], Sebastjan Radisek[2], Branka Javornik[1] [1]University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, Ljubljana, Slovenia [2]Slovenian Institute for Hop Research and Brewing, Cesta Zalskega tabora 2, Zalec, Slovenia e-mail: stanislav.mandelc@bf.uni-lj.si

Plant pathogenic fungi employ a broad range of extracellular proteins in the pathogenesis process and these proteins are often crucial to the success of the infection. The most important of these proteins are hydrolytic enzymes, which degrade physical barriers (cuticle, cell walls), specific toxins and effectors, which cause changes in plants that facilitate infection. Verticillium wilt of hop is a vascular wilt disease resulting from the colonization of xylem vessels by *Verticillium albo- atrum*. Fungal isolates differ in agressiveness and have been classified by pathogenicity tests into mild and lethal pathotypes. In the present study, we cultured six isolates (belonging to mild and lethal pathotypes and originating from three countries) in a simulated xylem medium to mimic *in vivo* nutritional conditions and therefore induce the secretion of proteins that are relevant to plant-pathogen interaction. Secreted proteins were concentrated from the culture supernatants and analyzed by two-dimensional difference gel electrophoresis (2D-DIGE). Approximately 850 proteins were detected in the samples, of which 275 were subjected to identification by mass spectrometry. The results will be presented in terms of secretome characterization and comparison between the isolates.

527. Polarity proteins Bem1 and Cdc24 are components of the filamentous fungal NADPH oxidase complex. Daigo Takemoto[1,2], Sachiko Kamakura[3], Sanjay Saikia[2], Yvonne Becker[2], Ruth Wrenn[2], Aiko Tanaka[1,2], Hideki Sumimoto[3] and Barry Scott[2] [1] Graduate School of Biogricultural Sciences, Nagoya University, Nagoya, Japan. [2] Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand. [3] Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Endophytic fungi *Epichloë festucae* systemically colonize the intercellular spaces of perennial ryegrass to establish a symbiotic association. We have shown that reactive oxygen species (ROS) produced by a specific NADPH oxidase isoform, NoxA, a regulatory component, NoxR, and the small GTPase, RacA have a critical role in regulating hyphal growth in the host plant. Generation of ROS by *E. festucae*, requires functional assembly of a multi-subunit complex composed of NoxA, NoxR, and RacA. However, the mechanism for assembly and activation of this complex at the plasma membrane is unknown. We found that *E. festucae* NoxR interacts with homologs of the yeast polarity proteins, Bem1 and Cdc24, and that the PB1 protein domains found in these proteins are essential for these interactions. GFP fusions of BemA, Cdc24 and NoxR preferentially localized to actively growing hyphal tips and to septa. These proteins preferentially interact with each other *in vivo* at these same cellular sites as shown by bimolecular fluorescent complementation assays. The PB1 domain of NoxR is essential for localization to the hyphal tip. An *E. festucae bemA* mutant was defective in hyphal morphogenesis and growth in culture and *in planta*. The changes in fungal growth *in planta* resulted in a defective symbiotic interaction phenotype. These results demonstrate that BemA and Cdc24 play a critical role in localizing Nox proteins to sites of fungal hyphal morphogenesis and growth.

528. Functional analysis of Six proteins: effectors of *Fusarium oxysporum*. Petra Houterman, Fabiano Sillo, Keigo Inami, Myriam Clavijo Ortiz, Ines Schreiver, Caroline Michielse, Ringo van Wijk, Fleur Gawehns, Lisong Ma, Mara de Sain, Frank Takken, Martijn Rep and Ben Cornelissen Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, P.O. Box 94215, 1090 GE Amsterdam, The Netherlands

The plant xylem-colonizing fungus *Fusarium oxysporum* f.sp. *lycopersici* (Fol) secretes small proteins into xylem sap during colonization of its host, tomato. We call these small proteins 'Six' proteins for '**S**ecreted **i**n **x**ylem'. Through gene knock-out and complementation we established that several Six proteins are required for full virulence. In addition, three Six proteins trigger R gene-dependent immunity and are therefore called Avr1, Avr2 and Avr3. Some *SIX* genes are activated specifically upon entry into roots, and expression of all *SIX* genes investigated is fully dependent on the transcription factor Sge1 ('**S**ix **g**ene **e**xpression **1**'). In accordance with this, strains deleted for *SGE1* are non-pathogenic and fail to invade tomato roots. Results of transient expression assays in leaves of *Nicotiana benthamiana* suggest that some Six proteins can suppress disease resistance reactions (hypersensitive response) while others enhance these reactions. Our aim is to uncover the molecular mechanisms underlying these effects. Ongoing work on the functional analysis of the Six proteins will be presented.

529. The signaling mucin Msb2 is processed into cellular and extracellular fragments during its function in appressorium formation of *Ustilago maydis*. Daniel Lanver, Patrick Berndt and Regine Kahmann. Max-Planck-Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl-von-Frisch-Strasse 10, D-35043 Marburg, Germany. daniel.lanver@mpi-marburg.mpg.de

The dimorphic fungus *Ustilago maydis* switches from budding to hyphal growth on the plant surface. In response to hydrophobicity and hydroxy fatty acids *U. maydis* develops infection structures called appressoria. Here we report on the transmembrane mucin Msb2, which is essential for appressorium formation in response to the hydrophobic stimulus. The Msb2-protein is processed into a cellular fragment and a large, highly glycosylated extracellular part. The latter fragment remains attached to the cell surface, as demonstrated by immunofluorescence and western analysis, but is also shed from cells. Although deletion of either part of Msb2 does not alter localization of the remaining part, both, the extracellular and intracellular domains are essential for function. We propose that the cellular part of Msb2 plays a role in activating downstream signaling events. Epistasis analysis revealed that Msb2 acts upstream of Kpp2 and Kpp6, two MAP-kinases essential for plant cuticle penetration. Collectively, our data indicates that Msb2 in *U. maydis* is a plasma membrane receptor involved in plant surface sensing. To reveal the impact of the Msb2-protein on gene regulation we performed genome-wide transcriptional profiling at the stage of appressorium formation and will discuss these results.

530. *Trichophyton rubrum* transcriptome in response to the cytotoxic drug Acriflavin measured by quantitative RNA sequencing. Gabriela Persinoti, Nalu Peres , Ricardo Vêncio, Nilce Martinez-Rossi Medical School of Ribeirão Preto, University of São Paulo, Brazil

Trichophyton rubrum is the most common etiologic agent isolated in cases of human dermatophytoses. Recently, it has become the cause of deep and widespread infections in immunocompromised patients. The therapeutic strategies to control thise infections have several limitations such as the appearance of resistant strains and the restricted number of cellular targets. New therapeutic strategies are necessary, being the focus of many investigations. Acriflavin is a cytotoxic drug with antifungal activity involved in topoisomerase inhibition. Although it is a DNA intercalate compound it has been reported the over-expression of genes coding for enzymes involved in mitochondrial respiratory-electron transport and in iron transport, suggesting a broad spectra of cellular effects. In order to better understand its molecular effects we are evaluating *T. rubrum* transcriptome in response to Acriflavin in a time-course assay using the next generation sequencing technology SOLID System. RNAseq was performed comparing *T. rubrum* growth in malt extract medium and the three periods of drug exposure. The differentially expressed genes will yield information about stress adaptation mechanism in dermatophytes, revealing new molecular targets for the development of novel therapeutic approaches and might also contribute to guide possible changes in the chemical structure of this drug, making it less toxic to humans, but still effective against fungi.

531. Characterization of *Trichoderma* isolates of marine origin and assessment of their potential as biocontrol agents. Inbal Gal-Hemed[1], Lea Atanasova[2], Monika Komon-Zelazowska[2], Irina S. Druzhinina[2], Ada Viterbo[1] and Oded Yarden[1] [1]The Hebrew Univ. of Jerusalem, Israel and [2]Inst. of Chemical Engineering, Austria

Fungi from marine origin are an appealing source due to their potential in either ecological or pharmaceutical aspects. *Trichoderma* spp. were isolated from the Mediterranean sponge *Psammocinia* sp. Identification of *Trichoderma* isolates was performed on the basis internal transcribed spacers 1 and 2 of rRNA, the large intron of translation elongation factor 1-alpha (*tef1*) gene, partial calmodulin (*cal1*) sequence and the RNA- polymerase subunit B II (*rpb2*)] using the on-line sequence similarity search tool *Tricho*BLAST and the identification program OKEY (www.isth.info). *Trichoderma* isolates, representing *T.* sect. *Trichoderma*, *T.* sect. *Pachybasium* and *T.* sect. *Longibrachiatum* were screened for tolerance to increasing osmotic pressure and temperature while carbon assimilation was investigated using Phenotype Microarray technique with BioLog TM FF MicroPlates. Isolates were screened for their antagonistic activity (antibiosis, secreted metabolites, mycoparasitsm) towards several plant pathogens. Two marine isolates were effective as biocontrol agents in greenhouse experiments against *Rhizoctonia solani* and due to their capability to colonize plant roots may be good candidates for inducing plant defense responses.

532. Origin of the plant-pathogen fungus *Verticillium longisporum* and insights into the interaction with its host *Brassica napus*. Susanna A. Braus-Stromeyer[1], Van Tuan Tran[1], Christian Timpner[1], Seema Singh[1,2], Oliver Valerius[1], and Gerhard H. Braus[1] [1] Georg August University, Goettingen, Germany [2] Maine Medical Center, Portland, ME, United States

Verticillium longisporum is a soil-borne fungal pathogen of oilseed rape (*Brassica napus*). The first research focus is the initial contact of the fungus to the plant which is the adhesion to host-plant roots. Non-adhesive yeast strains were used to screen for candidate genes for adhesion of *V. longisporum*. 28 adhesive candidate genes are currently under investigation for their importance in the infection process. We further investigated the reaction of the fungus to xylem sap of the host-plant by differential expression of proteins related to reactive oxygen stress. We could identify a catalase-peroxidase encoded by two highly similar isogenes of *V. longisporum*. It is induced by the presence of xylem sap and is required for the late phase of disease development. The isolation of additional isogene pairs hints to the evolutionary origin of *V. longisporum*, which is still controversially discussed. It is distinct from both *V. dahliae* and *V. albo-atrum* by possessing some typical characteristics such as long spores, almost double amount of nuclear DNA content and cruciferous host specificity. *V. longisporum* is an example for an early stage of speciation of the *V. dahliae* and *V. albo-atrum* interhybrid.

533. Genome wide analysis of the secretome repertoire in the poplar rust pathogen *Melampsora larici-populina*. Sébastien Duplessis[1], Stéphane Hacquard[1], David L Joly[2], Yao-Cheng Lin[3] and The Melampsora Genome Consortium[1,2,3,4] [1]UMR 1136 IAM, INRA Nancy, Champenoux, France. [2]Canadian Forest Service, Laurentian Forestry Centre, Québec, Canada. [3]Department of Plant Systems Biology, VIB, Ghent, Belgium. [4]DOE Joint Genome Institute, Walnut Creek, CA, USA.

The foliar rust caused by *Melampsora larici-populina* (Mlp) is a major disease affecting poplar plantations with severe economic losses. In the wake of the *Populus* genome project, the sequencing of the ~100 Mb genome of *Mlp* is a great opportunity to identify loci coding for the arsenal developed by the rust fungus to penetrate and exploit its host. The annotation of the genome identified homologs of previously described haustorially expressed genes including rust avirulence factors and many *Mlp* specific small secreted proteins (SSPs). Analysis of clusters of paralogous genes revealed candidate effectors under diversifying selection. Transcript profiling of *Mlp* SSPs showed timely coordinated expression at different stages of plant infection, most being expressed after the formation of haustoria. Immunolocalization validated the accumulation of some candidate effectors in rust haustoria and infection hyphae. Brought together, these analyses help in prioritizing candidate effector genes likely involved in coevolutionary interplay between the pathogen and the plant immune system for functional studies. *The Mlp genome was sequenced by the US Department of Energy Joint Genome Institute*

534. Withdrawn

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

535. Withdrawn

536. Protein Interaction Analysis of AVR-Pia of *Magnaporthe oryzae* by Yeast Two Hybrid Assay. Yuki Satoh[1], Shinsuke Miki[1], Toyoyuki Ose[2], Yudai Okuyama [3], Hiroyuki Kanzaki[3], Ryouhei Terauchi[3] and Teruo Sone [1 1] Graduate school of Agriculture, and [2] Research faculty of Pharmacology, Hokkaido University, Sapporo Japan. [3] Iwate Biotechnology Research Center, Iwate Japan. yuki- s@chem.agr.hokudai.ac.jp

The avirulence gene *AVR-Pia*, which induces HR of rice cultivars with the resistance gene *Pia* was isolated from *Magnaporthe oryzae* strain Ina168. *AVR-Pia* is 255-bp encoding 85 amino acids, including N-terminal 19 amino acids which are predicted to be signal peptides. Two resistance gene analogs *RGA4* and *RGA5* which have a nucleotide binding site and a leucine rich repeat (NBS-LRR), are located on *Oryza sativa Pia* locus and required for the function as *Pia*. Interaction of proteins encoded by these genes was investigated in this study. The interaction between AVR-Pia, RGA4 and/or RGA5 was analyzed by using Matchmaker Gold Two Hybrid system (Clontech). Native AVR-Pia, AVR-Pia without signal peptide (AVR-Pia-sp), RGA4 and RGA5 were expressed independently in the yeast cells. The interaction between those proteins was investigated by reporter gene expression after cell mating. All reporter genes were expressed in the mated cells between two AVR-Pia-sp. However, the interaction in other combination mating was not observed although the expression of proteins in the yeast cells was confirmed by the Western blotting. Therefore there is a possibility that AVR-Pia has the interaction between itself and multimers of AVR-Pia participates in the plant cells during the infection. Modified AVR-Pia proteins were constructed by, i) C terminal truncation and ii) substitution of two cysteine residues at 25th and/or 66th by glycine. These modifications caused the loss of function of AVR-Pia in inoculation assay, and interaction between modified AVR-Pia and itself was found to be relatively weak. These results suggest that the formation of AVR-Pia homo multimers is related to the function of AVR-Pia protein.

537. *In planta* expression of *Magnaporthe oryzae AVR-Pia* gene. Teruo Sone[1], Shinsuke Miki[1], Yuki Satoh[1], and Toyoyuki Ose[2],[1]Research Faculty of Agriculture and [2]Research Faculty of Pharmacology, Hokkaido University, Sapporo Japan. sonet@chem.agr.hokudai.ac.jp

AVR-Pia is one of the *Magnaporthe oryzae* AVR genes and was cloned from strain Ina168 (Miki et al., 2009). *AVR-Pia* is 255 bp in length and encodes 85 aa with 19aa-secretion signal, but its function is still unknown. In this study, *in planta* expression profile of *AVR-Pia* was investigated using qRT-PCR of inoculated rice leaf and fluorescent microscopy of rice leaf sheath inoculated by *M. oryzae* expressing GFP-tagged AVR-Pia protein. Strain Ina168 was inoculated onto either compatible or incompatible rice. Inoculated leaves were harvested in a time- dependent manner and total RNA was immediately extracted from them. The *AVR-Pia* expression was then analyzed by qRT-PCR. On both inoculation assays, the expression was first detected at 24 hours after inoculation (hai). After that, on compatible rice, the expression was increased until 60 hai, whereas it was reduced after 30 hai on incompatible rice. Ina168m95-1 transformed by a vector with AVR-Pia::EGFP showed the incompatibility toward rice cultivar with *Pia* gene, indicating GFP-tagged AVR-Pia complemented the function of AVR-Pia. The expression of AVR-Pia::EGFP was successfully detected during infection of intact leaf sheath of compatible rice cultivar. EGFP was accumulated at BIC (biotrophic interfacial complex, Khang et al., 2010)-like structure of invasive hyphae. These results suggests the function of AVR-Pia as invasive effectors.

538. A Mass Spectrometry Based Examination of the Magnaporthe oryzae Proteome During Appressorium Development. William L Franck[1], Emine Gokce[2], Yeonyee Oh[1], Timothy S. Collier[2], David C. Muddiman[2] and Ralph A. Dean[1] [1]Center for Integrated Fungal Research, [2]W.M. Keck FT-ICR Mass Spectrometry Laboratory, Department of Chemistry, North Carolina State University, Raleigh, North Carolina, 27606

The rice blast pathogen, *Magnaporthe oryzae*, penetrates leaf surfaces via infection structures known as appressoria. Appressorium formation can be induced by germination of conidia on hydrophobic surfaces or hydrophilic surfaces in the presence of cAMP. To better understand the physiological changes occurring during the formation of appressoria, a mass spectrometry-based proteomics study was initiated to examine changes in the proteome during spore germination and appressorium development. 1393 proteins were identified from five conditions including, conidia and conidia germinated on a hydrophilic surface for 4 and 18 hours in the presence or absence of 50mM cAMP. A detailed examination of this data including changes in protein abundance will be presented. In addition, an analysis of the phosphoproteome is in progress. Protein kinase dependent signaling is indispensible for production of appressoria. Phospho-(Ser/Thr) kinase substrate antibodies were used to observe kinase-specific changes in protein phosphorylation patterns between mycelium, conidia and germinated conidia forming appressoria. These results provide a foundation for examining the phosphoproteome and identifying protein kinase targets involved in appressorium development.

539. Deep RNA sequencing improved the structural annotation of the *Tuber melanosporum* transcriptome. E. Tisserant [1], C. Da Silva [2], A. Kohler [1], E. Morin [1], P. Wincker [2], and F. Martin [1 1] INRA, UMR INRA/Nancy Université 'Interactions Arbres/Micro-Organismes', INRA-Nancy, 54280 Champenoux, France; [2] CEA, IG, Genoscope, 2 rue Gaston Crémieux CP5702, F-91057 Evry, France

Although the analysis of the genome of the Perigord Black truffle (*Tuber melanosporum* Vittad.) has revealed unique features, the functional complexity of its transcriptome has not yet been fully elucidated. Here, we applied high- throughput Illumina RNA-sequencing (RNA-Seq) to the transcriptome of T. melanosporum under different major developmental stages, i.e. free-living mycelium, fruiting body and ectomycorrhiza. Sequencing of cDNA libraries generated a total of ~24 millions sequence reads representing more than 882 Mb of sequence data. To construct a coverage signal profile across the genome, all reads were then aligned to the reference genome assembly of T. melanosporum Mel28. We were able to identify a substantial number of novel transcripts, antisens transcripts, new exons, UTRs, alternative upstream initiation codons and upstream open reading frames. This RNA-Seq analysis allowed us to improve the genome annotation. It also provided us with a genome-wide view of the transcriptional and post- transcriptional mechanisms generating a increased number of transcript isoforms during major developmental transitions in *T. melanosporum* .

540. Lifestyle, genetic diversity and host-pathogen interactions of *Ascosphaera*. Annette B. Jensen[1], Doris Roth[2], Lene Lange[2], Jacobus Boomsma[3], Jørgen Eilenberg[1]. [1]Department of Agriculture and Ecology, University of Copenhagen, Denmark, [2]Section for Sustainable Biotechnology, Aalborg University, Ballerup, Denmark, [3]Department of Biology, University of Copenhagen, Denmark.

Fungi from the genus *Ascosphaera* (Ascosphaeraceae, Onygenales) are all associated with bees, social bees like honey bees or solitary bees like leafcutter bees. Some of the species are pathogens infecting bee larvae through the gut causing the chalkbrood disease while others are saprophytes using pollen provisions or other nest material as substrates. Trypsin-like peptidases are expected to represent markers of fungal pathogenicity and serine peptidase, a subgroup of trypsin-like peptidases, has been found in the genome of *Ascosphaera apis*. We will look for serine peptidase genes in various *Ascosphaera* species to test the marker-hypothesis on closely related species. In the battle between host and pathogen several excreted enzymes and peptides are involved and we are currently applying TAST (transposon-assisted signal trapping) (Becker et al. (2004) J Microbiol Methods 57:123-133) to explore this interaction further in detail for the *A. apis* -honey bee system. *Ascosphaera apis* is an obligate pathogen of honey bees and it occurs almost world wide as the distribution of its host. We have examined the genetic diversity and population structure of strains collected throughout Europe.

541. Dissecting *Colletotrichum higginsianum*'s response to osmotic stress. Stefan G Amyotte, Saurabh Kulshreshta, Patrick Dotson, Bidisha Chunda, Srivathsa C. Venugopal, Ken-Taro Sekine, Aardra Kachroo, Pradeep Kachroo, and Lisa Vaillancourt University of Kentucky, Lexington, KY

Glycerol-3-phosphate (G3P) is an important component of carbohydrate and lipid metabolism. Deletion of the cytoplasmic G3P dehydrogenase (G3Pdh) in *Colletotrichum higginsianum* resulted in reduced levels of glycerol and G3P. The mutants have cell wall defects, reduced appressorial turgor, and significant alterations in fatty acid metabolism. In culture, the mutants do not grow normally unless the media is amended with glycerol, or interestingly, high levels of an osmoticum (NaCl or Sorbitol). There are two possibilities to explain how osmotic stress complements the growth defect. First, osmotic stress may cause accumulation of glycerol as a compatible solute, and this may be converted to G3P and thus complement the phenotype. The second possibility is that osmotic stress results in alterations in the level of saturation of membrane fatty acids, and that this restores normal growth in culture. To test these alternate hypotheses, we are characterizing polyols that would serve as compatible solutes, as well as the fatty acid composition of the wild-type and mutants upon exposure to NaCl or sorbitol. The G3Pdh mutants also had a significant reduction in pathogenicity to wounded or unwounded Arabidopsis leaves. We are tracking the production of fluorescent fusion proteins in order to monitor the expression of genes involved in glycerol metabolism and membrane lipid composition, as well as other genes involved in the response to osmotic stress *in planta*.

542. Exploring the symptomless phase of *Fusarium graminearum* infection of wheat ears. Neil A. Brown, Martin Urban and Kim E. Hammond-Kosack Dept of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK neil-a.brown@bbsrc.ac.uk

Fusarium Ear Blight is a devastating disease of all cereals. Besides the reduction in crop yield and quality, the grain becomes contaminated with various harmful mycotoxins. Annually this disease causes millions of dollars in crop losses worldwide. A study of the infection biology of *Fusarium graminearum*, using light and electron microscopy, revealed the existence of a substantial phase of symptomless wheat ear infection and that hyphae establish infection by colonising the intercellular space between live plant cells (Brown *et al*., (2010) Fungal Biology). Importantly, an RT-qPCR analysis using a newly devised linear rachis infection bioassay revealed that the expression of various genes, essential for mycotoxin production i.e. *TRI4*, *TRI5*, *TRI9* and *TRI14*, is elevated up to 11 fold at the advancing hyphal front. Conversely, *GAPDH* expression dropped 7 fold in this tissue. This study supports the hypothesis that the mycotoxin deoxynivalenol plays a role in inhibiting plant defences, enabling symptomless infection (Brown *et al*., (2011) submitted). To further understand how this destructive pathogen modulates its growth and development during key points in the ear infection process, specific host cell-types are being isolated by laser capture micro-dissection. This will enable a detailed spatial and temporal exploration of fungal gene expression *in planta*. An analysis of the *Fusarium* secretome will also provide clues into how the fungus manipulates the host environment to permit symptomless infection.

543. Global gene expression studies of *Fusarium graminearum* during the infection process of wheat and barley. Erik Lysøe[1], Kye-yong Seong[2], H. Corby Kistler[2]. [1]Bioforsk, Ås, Norway. [2]University of Minnesota/Cereal Disease Laboratory, St. Paul, USA.

Fusarium graminearum causes head blight disease in wheat, and to help understand the infection process we studied global gene expression of *F. graminearum* in a time series of 24 to 196 hours after inoculation, compared to a water control. The infection is rapid and already after 48h over 4000 genes is expressed. The number of genes expressed increased over time up to 96h (>8000 genes), and went down again 144h and 192h post inoculation. After subtraction of genes found expressed on complete media, during C and N starvation, and on barley, only 355 were found exclusively expressed in wheat, mostly ones with unknown function (72,6%). These were mainly found in SNP enriched islands on the chromosomes, suggesting a higher evolutionary selection pressure of wheat specific genes. The annotated genes found were enriched in functional groups involved in allantoin and allantoate transport, detoxification, nitrogen, sulfur and selenium metabolism, secondary metabolism, carbohydrate metabolism and degradation of polysaccharides and ester compounds. Several putative secreted virulence factors were also found expressed in wheat.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

544. Characterization of a Peroxisomal Protein Complex Regulating Stalk Rot Virulence in *Fusarium verticillioides*. Jung-Eun Kim, Chih-Li Wang, Brian D. Shaw, and Won-Bo Shim. Department of Plant Pathology & Microbiology, Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX 77843-2132

Fusarium verticillioides is one of the key stalk rot pathogens on maize. Previously, we discovered that Fsr1 protein plays a key role in stalk rot virulence. The predicted Fsr1 has multiple protein-binding domains, and the coiled-coil (CC) domain in the N-terminus was determined critical for virulence in *F. verticillioides*. The CC domain is known to mediate protein-protein interactions in many eukaryotes, and our premise is that this interaction triggers downstream gene signaling associated with stalk rot virulence in *F. verticillioides*. The aim of this study was to identify putative Fsr1-binding proteins and determine their role in stalk rot virulence. We performed yeast two-hybrid experiments using the N-terminus region of Fsr1 as bait and found two putative proteins binding to the CC domain of Fsr1 in *F. verticillioides*: Wor1 and Pex14. We further verified these interactions through co-immunoprecipitation. *In silico* analyses revealed that both Wor1 and Pex14 shares high similarity with peroxisomal proteins in fungi. For further characterization, we generated *wor1* and *pex14* deletion mutants. Interestingly, while both mutants showed reduced stalk rot virulence, they were not as severe as the symptom caused by the *fsr1* deletion. Our data suggest that peroxisomal proteins, Wor1 and Pex14, interact with Fsr1 to regulate stalk rot virulence in *F. verticillioide*s. We are currently generating double deletion mutants to test virulence on maize. Further *in viv*o confirmation of Fsr1-Wor1/Pex14 interactions with the use of fluorescent proteins is also in progress.

545. Expression of recombinant fungal proteins with potential relationship to fungal allergy. Torrecilla, Ignacio[1]; Lawrence, Christopher B[2]; Dunn-Coleman, Nigel S[1]; and Díaz-Torres, María R. [1] [1]AlerGenetica SL, Santa Cruz de Tenerife, Spain. www.alergenetica.com [2]Virginia Bioinformatics Institute, Blacksburg, USA.

Allergies are the fourth most common disease in the world with between 40-50% of the World's population affected. A substantial portion of this population (10-30%) is allergic to filamentous fungi present in the environment with patients suffering from allergic rhinitis to more severe cases of asthma. At present there is no curative treatment for fungal allergy. AlerGenetica, in collaboration with the Universities of Barcelona and Manchester, intends to profile which fungal allergens patients are sensitized to. The initial focus of the research is profiling patients sensitized to allergens from *Aspergillus fumigatus* and *Alternaria alternata*. In order to express these allergens, we have selected two expression systems: *Pichia pastoris*, and *Aspergillus niger*. *This research is partially funded by the Torres Quevedo Program of the Spanish Science and Innovation Department*.

546. The role of the striatin ortholog in virulence, conidiation, and sexual development of *Colletotrichum graminicola*. Chih-Li Wang, Won-Bo Shim, and Brian D. Shaw Dept. Plant Pathology and Microbiology, Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX 77843-2132, USA

Colletotrichum graminicola is a foliar pathogen of maize causing anthracnose and stalk rot. In filamentous fungi, striatin orthologs have been implicated in multiple developmental processes including sexual development and pathogenicity, and localize to the endoplasmic reticulum and the nuclear envelope. However, little is known about its role in *C. graminicola*, a heterothallic, hemi-biotrophic pathogen of maize. To characterize the gene in *C. graminicola*, we generated *str1* deletion mutants using a split-marker recombination strategy. Mutants grew slower on nutrient media, and lost the clockwise spiral growth typically observed in wild type on PDA. Interestingly, the mutants showed a counter-clockwise spiral growth pattern on V8 medium. The *str1* mutants produced shorter falcate conidia and fewer, less developed acervuli on nutrient medium and autoclaved maize leaf, resulting in reduced falcate conidium production. Oval conidium production was low. When crossed with sexually compatible strains, the *str1* mutants were not capable of producing perithecia on autoclaved maize leaves. The mutants still produced appressoria and successfully penetrated leaves, but were delayed in foliar lesion development. In stalk rot assays, mutants produced reduced lesions at the primary infection sites. Our study indicated that the *str1* deletion mutants were defective in several propagation-associated developmental events and were less virulent to maize.

547. Remodeling of the fungal cell wall contributes to Fludioxonil and Ambruticin resistance in the dermatophyte *Trichophyton rubrum*. Nalu Peres[1], Diana Gras[1], Pablo Sanches[1], Antonio Rossi[1], Rolf Prade[2], Nilce Martinez-Rossi [1]. [1]University of Sao Paulo - Brazil, [2]Oklahoma State University - USA. e-mail: nalu@usp.br

Fungal infections have become a health problem worldwide, leading to the need for the development of new efficient antifungal agents. Although dermatophytes do not cause life-threatening diseases, there are reports of deep infections and severe lesions in immunossupressed patients and impairment of living standards of the infected individuals. The antifungal compounds Fludioxonil and Ambruticin present a unique mode of action, interfering with the fungal osmotic signaling pathway. We evaluated the effect of these drugs on *Trichophyton rubrum*, and low doses of the drugs inhibit growth of this dermatophyte, leading to hyphal-tip swelling, rupture of cell wall, and leakage of cell contents. We isolated Ambruticin/Fludioxonil resistant mutants with UV, which showed enhanced conidiation, altered pigmentation, modified vegetative growth rates, and higher sensitivity to osmotic stress. Using the sib-selection approach we isolated two genes encoding a phospholipid transporter and the glucan 1,3- beta glucosidase protein, which conferred resistance to Ambruticin and Fludioxonil to the wild type strain. Since these enzymes are involved in the remodeling of fungal cell wall, we suggest that this process may be an important mechanism contributing to the resistance to both drugs. Financial Support: FAPESP, and CNPq.

548. Connecting the tetraspanin BcPls1 of *Botrytis cinerea* to the Nox complex. Jens Heller, Ulrike Siegmund, Stefan Mester and Paul Tudzynski IBBP, WWU Münster, Schlossgarten 3, D-48149 Münster, Germany jens.heller@uni-muenster.de

Animal tetraspanins are membrane proteins controlling cell adhesion, morphology and motility. In *Magnaporthe grisea* the tetraspanin MgPls1 was first identified as a virulence factor by plasmid-mediated insertional mutagenesis. In this hemibiotrophic fungus MgPls1 controls an appressorial function required for the penetration into host plants. In the strain T4 of the necrotrophic fungus *Botrytis cinerea* a similar effect for Pls1 has been observed. In this strain deletion mutants of *bcpls1* are apathogenic on unwounded host tissue due to formation of non-functional appressoria. We performed a functional characterization of *bcpls1* in the more aggressive *B. cinerea* strain B05.10. The growth behavior of the deletion mutant in axenic culture does not differ from the wildtype. Unlike the deletion mutant of the strain T4, the B05.10 derived mutant is still able to infect unwounded bean leaves, although infection is delayed. However, penetration assays on onion epidermis revealed that the mutant is impaired in appressoria-mediated penetration. Since this phenotype is very similar to that of the *bcnoxB* deletion mutant a connection between BcPls1 and BcNoxB is hypothized. A functional GFP-BcPls1 fusion protein is detected in appressoria where it is localized in membrane structures and vacuoles. We conclude that the membrane protein BcPls1 is required to define localization of BcNoxB during infection.

549. From Yaps to Cap and their role in fungal pathogenesis. Charu Jain, Kelly Pastor, Reeta Prusty Rao. Department of Biology and Biotechnology, Gateway Park, WPI, Worcester, MA-01605. charu@wpi.edu

Hospital acquired fungal infections in immune-compromised patients; primarily due to *Candida albicans*, has become more prevalent in the last decade. Increasing resistance to the available antifungals and lack of new drugs has added to the problem of treatment. We have developed an *in vivo* pathogenesis assay using *C. elegans* as the model host to better understand the infection process as well as identify novel drug targets. The assay is amenable to high-throughput screening protocols because it involves co-culturing egg preparations from worms in a mixture of *E. coli* and Candida allowing us to screen Candida mutant libraries to identify fungal virulence factors. Host factors that confer resistance can also be identified using RNAi libraries introduced into the *E. coli*. Using this assay we show that *C. albicans* AP-1 transcription factor, Cap1, is required for virulence. Worms exposed to the *cap1*$^{-}$*/-* survive longer than those exposed to wild type candida. However if the host's ability to produce the oxidative environment is impaired, the *cap1*$^{-}$*/-* is able to infect suggesting that Cap1 is involved in neutralizing ROS. Finally we demonstrate that wild type candida is able to lyse macrophages and escape killing while the *cap1*$^{-}$*/-* mutant cannot. Cap1, a fungal specific transcription factor is a likely target for development of antifungal drugs.

550. Fungal inhabitants of the healthy mammalian gastrointestinal tract. Heather E. Hallen-Adams Department of Food Science and Technology, University of Nebraska, Lincoln, NE 68583-0919, USA

Over the past decades, there has been a growing realization that organisms do not live in a vacuum; rather, complex organisms may best be thought of as communities and assemblages, spanning multiple Kingdoms and Domains of life. The mammalian gastrointestinal tract, providing warmth, shelter and nutrients, is home to an extensive microbial community. To date, most research has focused on characterizing the prokaryotic members of this community. Fungi have long been known to inhabit the mammalian digestive tract; however, the nature and composition of fungal GI tract communities has been little studied (with few exceptions, e.g. the rumen chytrids of cattle; and fungi associated with certain disease states). We have used fungal-specific ITS primers to perform 454 survey sequencing on DNA isolated from feces of healthy humans, mice and cattle in order to provide a baseline characterization of GI tract fungi. Fungi are present in all samples examined; the similarities and differences in fungal communities between and within species will be discussed, as will their potential importance to the host in terms of nutrient acquisition and utilization. We are just beginning to learn how the unique demands of life in the gastrointestinal tract (temperature > 36 C; anaerobic conditions; mass flow and muscular contractions) have shaped the ecology of these organisms.

551. Host-specific colonization of sorghum by Sporisorium reilianum depends on absence of the phytoalexin luteolinidine. Katja Zuther, Julia Imkampe, Simon Uhse, Jan Schirawski Georg-August-Universität Göttingen, Albrecht-von-Haller Institute, Molecular Biology of Plant-Microbe-Interactions, Untere Karspüle 2, 37073 Göttingen, Germany. jschira@uni-goettingen.de

The smut fungus *Sporisorium reilianum* occurs in two varieties (*S. reilianum f. sp. reilianum* [SRS] and *S. reilianum f. sp zeae* [SRZ]) that cause head smut disease on the economically important crops sorghum and maize, respectively. After plant penetration, fungal filaments penetrate the leaf surface, systemically spread throughout the plant and reach the inflorescences, in which spore formation occurs. To elucidate the basis of host specificity of the two *S. reilianum* varieties, we compared disease etiology of SRS and SRZ on sorghum and maize. Although SRS was only able to produce spores on sorghum, and SRZ generated spores exclusively on maize, both varieties were able to penetrate and multiply in both hosts. However, while an SRS infection of sorghum led to mild chlorosis on inoculated seedling leaves, sorghum infection with SRZ induced appearance of red spots that darkened over time and eventually led to death of colored plant cells. Using MALDI-TOF analysis of leaf extracts we show that sorghum reacts with the production of the red phytoalexin luteolinidine only after colonization by SRZ but not by SRS. Using growth assays we demonstrate that luteolinidine strongly inhibits growth of both SRS and SRZ. Interestingly, the luteolinidine biosynthesis gene *SbFNS2* is only induced upon plant penetration by SRZ. This suggests that luteolinidine production is essential to prevent colonization of sorghum by *S. reilianum*.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

552. Isolation of *Phoma tracheiphila* impaired pathogenicity mutants and study of *in planta* fungal progress. Tammy Kroitor-Keren and David Ezra Plant Pathology department, ARO,The Volcani Center,Bet Dagan,Israel E-Mail:dezra@volcani.agri.gov.il Mal

secco disease caused by the pathogenic fungus *Phoma tracheiphila* is one of the most devastating diseases of susceptible citrus species, particularly lemon. ATMT was used to transform *P.tracheiphila* phialoconidia with the binary vector pSK1019 containing the *E. coli* hygromycin phosphotransferase B resistance gene *hph*)under the *Aspergillus nidulans trpC* promoter and EGFP gene under a Ch GPD promoter. A rapid screening method was developed for evaluating pathogenicity and aggressiveness of the transformants. A pathogenic transformant expressing *gfp* activity was used to observe the *in planta* progress of the fungus following inoculation. A total of 2263 different transformants were generated for pathogenicity evaluation. Following four screening procedures, three were found to be non-pathogenic and 43 had reduced pathogenicity. Four mutants were further examined for insertion localization and characterization. Three had only one insertion while the fourth had multiple insertions. Using the Genome Walking method (Clontech), the sequences of two non-pathogenic mutants were determined. Computer analysis of the sequences showed: for mutant 3-117, a 2971bp long sequence coding for a hypothetical protein of 536aa. BLAST-X comparison to the gene bank displayed 50% identity to RNA-directed RNA polymerase 2 (RDRP) from *Phaeosphaeria nodorum*. The second mutant (1-241) possessed a 2031bp-long sequence coding for a hypothetical protein of 322aa. BLAST-X comparison to the gene bank displayed 94% identity of 170aa of this hypothetical protein to Intradiol dioxygenase from *Pyrenophora tritici-repentis*.

553. Different supernumerary chromosomes of *Nectria haematococca* can determine different habitat specificities. Gerard White[1], Marianela Rodriguez-Carres[2], and Hans VanEtten[1]. [1] School of Plant Science, University of Arizona, Tucson, AZ, USA. [2] Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA.

The habitat diversity of the fungus *Nectria haematococca* MPVI has been shown to be party due to conditionally dispensable (CD) chromosomes that carry habitat-defining genes. Previous studies showed that the *N. haematococca PDA1* CD chromosome, which carries genes for pea pathogenicity (*PEP*), also has genes for the utilization of homoserine (*HUT*), an amino acid in pea root exudates. We identified a cluster of five genes (*HUT* cluster) on the *PDA1* CD chromosome that was responsible for the HUT phenotype in *N. haematococca*. Competition experiments comparing HUT^+ and HUT^- isolates showed that the *HUT* cluster confers increased competitive ability to HUT^+ *N. haematococca* isolates in the rhizosphere of pea. Competition studies using fluorescent protein-tagged HUT^+ and HUT^- isolates supported that finding. The *HUT* cluster is not present on the *PDA6* CD chromosome and further analyses have demonstrated that the two different CD chromosomes confer competitive advantage in the rhizospheres of different plant species. Both the *PDA1* and *PDA6* CD chromosomes confer a competitive advantage in the rhizosphere of soybean, whereas only the *PDA6* CD chromosome confers a competitive advantage in the rhizospheres of tomato and alfalfa, and only the *PDA1* CD chromosome confers a competitive advantage in the rhizosphere of pea. These competition studies, combined with analyses showing that approximately two-thirds of the DNA is unique to each chromosome, suggest the presence of unique genes on the *PDA6* and *PDA1* CD chromosomes that allow for an expanded habitat of *N. haematococca*. Preliminary sequencing data support this hypothesis. These *N. haematococca* CD chromosomes appear to be analogous to host-specifying plasmids in plant-associated bacteria.

554. Detection by proteomics of small secreted proteins in a *Trichoderma virens* / maize interaction. Netta Dahan-Lamdan and Benjamin A. Horwitz, Department of Biology, Technion, Haifa, Israel

Small, cysteine rich secreted proteins (SSPs) play important roles in fungal-plant interactions. Searching the *Trichoderma virens* genome revealed over 200 SSP genes, including Sm1 [1], and previously known hydrophobin genes. To study the SSP protein expression pattern, we performed secretome analysis of T.virens growing by itself or with maize roots. Medium from maize plants growing in hydroponic culture with or without *T. virens* was concentrated on a Q-Sepharose column and eluted with 1 M NaCl. We used peptide dimethylation with isotopically coded formaldehyde labeling trypsin digest peptides. The peptides were analyzed by LC-MSMS. Data analysis was done vs the Joint Genome Institute *T. virens* database. In two repeats a similar number of proteins was detected (50 and 54) at high confidence, with 25 proteins common to the two experiments. SSPs made up 16% of the total secreted proteins. 13 SSPs were detected, all with homologs in *T. virens* and/or *T. atroviride* and *T. reesei*, except for one unique protein. Perhaps surprisingly, we have not detected proteins whose abundance is strongly increased by co-culture with maize roots. On the contrary, several proteins showed decreased abundance, suggesting that they might be sequestered by the roots. The secreted proteins are candidates for elicitors of systemic induced resistance; their roles can be studied by constructing loss of function and overexpressing lines. [1] Djonovic et al. (2007). Plant Physiol. 145:875-89.

555. Phenolic compounds as small molecule signals in *Cochliobolus heterostrophus*. Samer Shalaby, Olga Larkov & Benjamin A. Horwitz, Department of Biology, Technion, Haifa 32000, Israel

The transcription factor ChAP1 of *Cochliobolus heterostrophus* responds to oxidative stress by migration to the nucleus and activation of antioxidant genes. Phenolic and related compounds found naturally in the host also trigger nuclear localization of ChAP1, but without triggering a strong antioxidant response. We searched for genes whose expression is regulated by phenolic compounds and/or ChAP1. The *C. heterostrophus* genome contains a cluster of genes for metabolism of phenolics. Intradiol dioxygenase (*CCHD1*), was strongly induced by caffeic and coumaric acids. At high phenolic concentrations ChAP1 is needed for maximum *CCHD1* expression, but not at micromolar levels. The pathogen thus detects phenolics by at least two signaling pathways: one causing nuclear retention of ChAP1, and another triggering induction of *CCHD1* expression. Phenolics and related compounds could provide a ubiquitous plant-derived signal [1]. In structure-activity studies we found that the carboxylic group in the phenolic compound is necessary for induction of *CCHD1*; the hydroxyl in the positions para and meta to the carboxylic group is necessary for high induction of *CCHD1* (10-fold), whereas a methoxy group in the same positions results in lower or no induction. Varying the side chain of the aromatic ring, we found that only the carboxylic group is essential. [1] Shanmugam, Ronen, Shalaby, Larkov et al. (2010) Cell Microbiol. 12: 1421-34

556. Expresson of small secreted proteins in response to coculture of *Trichoderma virens* with maize roots. Naomi Trushina[1], Prasun K. Mukherjee[2,3], Mala Mukherjee[1], Charles M. Kenerley[3] and Benjamin A. Horwitz[1] [1]Department of Biology, Technion, Haifa, Israel, [2]BARC Mumbai India, [3]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA

Small secreted proteins (SSP) may have roles in the interaction of *Trichoderma* with plant roots, and with fungal hosts in mycoparasitic interactions occurring in the rhizosphere and in the soil. A large set of predicted SSPs was identified from the *T. virens* genome [1]. Most of these proteins have no known function, except for the elicitor gene *Sm1* [2]. To test the hypothesis that expression of SSP genes depends on signals generated by coculture with maize roots, we measured the relative transcript levels of a subset of SSPs in RNA isolated from T. virens grown in interaction with maize roots in hydroponic culture. For comparison, transcript levels were measured for RNA isolated from mycelia grown axenically. Some SSP genes were upregulated and others down-regulated in a series of semi-quantitative RT PCR experiments. We are currently exploring the expression of SSP genes on a larger scale using oligonucleotide microarrays. The results will help guide our choice of SSP genes for study for their possible roles in induced systemic resistance. [1] http://genome.jgi-psf.org/TriviGv29_8_2/TriviGv29_8_2.home.html [2] Djonovic et al. (2007). Plant Physiol. 145:875-89.

557. *NOX* genes of *Cochliobolus heterostrophus*: role in ROS production, development and virulence. Mordechai Ronen and Benjamin A. Horwitz, Department of Biology, Technion, Haifa 32000, Israel

Specific NADPH oxidases (*NOX*), enzymes inserted in the plasma membrane produce reactive oxygen species (ROS). NOX enzymes use cytoplasmic NADPH to produce superoxide. ROS have both defense and signaling roles. Fungal NADPH oxidases have a structure very similar to the human gp91phox. Specific isoforms of fungal NOX have been reported to be required for various physiological processes and cellular differentiation events, including development of sexual fruiting bodies, ascospore germination, and hyphal growth in both mutualistic and antagonistic plantâ€“fungal interactions. We identified three NADPH oxidase homologues in the necrotroph *Cochliobolus heterostrophus*, and investigated their function and importance in the fungal life cycle through study of loss-of-function mutants in genes encoding catalytic and regulatory subunits of NADPH oxidases. Mutants in *noxA*, *noxC* and the predicted regulatory subunit gene *noxR* have decreased pigmentation and delayed conidiation. *noxA*, *noxR* show decreased virulence on the host plant, maize. All nox mutants produce superoxide as detected by nitro blue tetrazolium (NBT) staining. Exposure of germinating conidia to maize extract increases ROS production, and this response is decreased in the triple *nox* mutant. We are following expression of oxidative stress-related genes in germinating conidia and during infection.

558. Microarray analysis of endophytic colonization of barley by the nematophagous fungus *Pochonia chlamydosporia.* Eduardo Larriba[1], Maria Jaime[2], Jose Martin-Nieto[1], Corey Nislow[2] and Luis V. Lopez-Llorca[1(*)] [1]Multidisciplinary Institute for Environmental Studies (MIES) "Ramon Margalef", University of Alicante, P.O. Box 99, E-03080 Alicante, Spain. (*) e-mail: lv.lopez@ua.es [2]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Pochonia chlamydosporia (Pc) is a nematophagus fungus with high potential as a biological control agent for nematodes. When developing endophytically, nematophagous fungi interact with monocot and dicot plants by eliciting defense responses and promoting plant growth. We have recently shown that Pc expresses serine proteases during endophytic colonization of barley (*Hordeum vulgare*) roots. Among these proteases, VCP1 is a main pathogenicity factor. We have also detected expression by Pc of a new class of fungal serine carboxypeptidase, SCP1, in such process. We have cloned, sequenced and addressed the expression of SCP1 and its implications in the biology of the fungus. The response of barley roots to endophytic colonization by Pc was analyzed transcriptomically using an Affymetrix Barley 1K array. Among the genes up-regulated by Pc some were involved in plant hormone signaling. Activation of auxin carriers and proteasome genes could explain the fungus-driven plant growth promotion found in bioassays. Also, activation of ethylene and jasmonic acid signaling pathways could be involved in priming, yet to be tested in bioassays. Down- regulation of plant genes such as beta-glucanase (a PR protein) or MYB transcription factor, reflected the non- pathogenic character of Pc. PRs (chitinases) and nematode resistance protein genes were instead up-regulated. Pc induced expression of numerous heat-shock protein genes in barley. Concluding, we have found genomic evidence for growth promotion and modulation of plant defenses by the nematophagous fungus Pc in barley. These results could have deep implications for sustainable disease control. We are currently validating our results by qRT-PCR and HPLC.

559. The relationship between mating type genes and pathogenicity in *Gibberella zeae.* Sladana Bec and Lisa Vaillancourt, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546, vaillan@uky.edu

Gibberella zeae (anamorph. *Fusarium graminearum*) is a primary causal agent of Fusarium Head Blight (FHB), a wheat disease responsible for major losses in North America and worldwide. *G. zeae* is a homothallic ascomycete capable of outcrossing. Ascospores serve as the primary source of inoculum in the FHB disease cycle. The sexual behavior of this fungus is regulated by the mating type locus *MAT1* , which is comprised of two idiomorphs, *MAT1-1* and *MAT1-2* . *MAT1-1* consists of three open reading frames (*MAT1-1-1*, *MAT1-1-2*, *MAT1-1-3*) and *MAT1-2* consists of a single open reading frame (*MAT1-2-1*). Previous research has shown that *G. zeae* can be transformed into an obligate heterothallic organism by deletion of either *MAT1-1-1* or *MAT1-2-1* , or into an asexual organism by deletion of the entire *MAT1* locus. In addition to their roles in self-non/self recognition and sexual development, as major transcriptional regulators mating type genes could have additional functions. We generated various MAT gene knockout strains in *G. zeae* PH-1, a lab strain with an available high quality genome sequence. The delta mat1-1-1 knockout strains showed a significant reduction in levels of aggressiveness on a susceptible winter wheat cultivar in the greenhouse, whereas there was a slight reduction in delta mat1-2-1 strains, and no significant reduction in delta mat1 whole-locus knockout mutants. These results suggest the interesting possibility that *MAT1-2-1* is a negative regulator of pathogenicity, and that interaction of the MAT1-2-1 protein with the MAT1-1-1 protein may mitigate this function.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

560. Association of *Fusarium graminearum* with root rots of edible legumes and gene expression during disease development. Rubella S. Goswami, Kishore Chittem, and William Yajima Dept. of Plant Pathology, North Dakota State University, Fargo, ND 58108-6050.

Fusarium species commonly associated with cereals, such as wheat and barley, are emerging as potential causal agents of root rots in edible legumes that are grown in rotation with these cereals. Extensive surveys of root rot affected dry bean and pea fields covering the major cereal and legume growing regions in North Dakota, demonstrates that *Fusarium* species such as *F. graminearum*, *F. culmorum*, *F. acuminatum*, *F. avenaceum* and *F. sporotrichioides* are present in infected roots along with, or in place of the traditional root rot pathogen *F. solani* and are capable of causing root rots on these legumes. Evaluation of the *F. graminerum* isolates from these surveys shows that they are capable of producing mycotoxins and infecting wheat heads. Gene expression studies using next generation sequencing approaches are being conducted to assess whether the pathways and genes associated with disease development are common between the cereal host, wheat and the leguminous host, field pea and between different types of disease reactions, namely, root rot and head blight. Initial findings have led to the identification of over eleven thousand *F. graminearum* genes that are expressed during infection of field pea roots, 6.57% of which are considered to be associated with cell defense, rescue and virulence. Several of these have been found to be similar to genes differentially expressed during development of crown rot in wheat, and those that are common between crown rot and head blight. Additionally, genes involved in the tricothecene pathway and those that are expressed by *F. avenaceum* , infecting field pea roots have also been detected. Various findings regarding pathogenicity and gene expression will be presented.

***561. Aspergillus* adhesins responsible for binding to human fibronectin.** Sungsu Lee, Sreevardhini Venkatramen, Diana Bartelt, Anne Dranginis. Dept. of Biological Sciences, St. John's University Queens, NY. 11439.

The first stage of Aspergillosis involves the binding of inhaled conidia to cell surface and extracellular matrix (ECM) proteins in the respiratory tract. Adherence of conidia of *A. fumigatus* to fibronectin (FN) has been demonstrated; however the molecular basis of interaction has not been clearly established. Identification of the fungal cell wall proteins will aid in the study of Aspergillosis and the development of potential drug targets. *A. nidulans*conidia bind to FN. We have used *S.cerevisiae* as a host to screen for *Aspergillus* adhesins. Binding of *S. cerevisiae* to FN is dependent upon expression of the cell surface flocculin Flo11/Muc1. A library of cDNA was prepared from conidiating cultures of *A. nidulans*. Several genes were identified *in silico*, including *MnpA* and *YpsA* and *A. fumigatus* genes *AFMP1* and *AFMP2*. The candidate genes and cDNA library were used to transform S288C*flo1¥Äflo11¥Ä*. Transformants were screened by binding to FN-coated magnetic beads and microtiter plates. Potential adhesion factors were identified by comparison with *Aspergillus spp.* DNA sequences in GenBank. Full length genes were cloned into a single copy vector with *GAL1* promoter. *MnpA, AFMP1, AFMP2* and *A. nidulans* hypothetical protein CBF76924.1 bound to FN. These may be *Aspergillus* adhesins involved in the interaction between conidia and human ECM. (Supported in part by NIGMS R15GM077345-01A1.)

562. The Mammalian Innate Immune Response to the Major Alternaria Allergen Alta1. Amanda Rumore[1,2], Shiv D. Kale[2], Mihaela Babiceanu[2], Ha X. Dang[2], Sang Wook Park[2], Brett M. Tyler[2], Hirohito Kita[3], and Christopher B. Lawrence[1,2] [1]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, USA, [2] Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA, [3]Mayo Allergic Diseases Laboratory, Mayo Medical School, Rochester, MN USA

Alta1 has been identified as an enzyme (esterase/phosphatase) and the major allergen secreted by *A. alternata* due to its IgE-specific reactivity with sera from atopic patients. No known innate immunological activity has been assigned to this clinically relevant protein. In order to elucidate the overall importance of Alta1 in pro-inflammatory cytokine production and allergic disease, we utilized a recombinant Alta1 protein, an Alta1 mutant protein lacking enzymatic activity, and an *A. alternata* mutant deleted for the Alta1 gene in human airway epithelial cell and mouse model studies. Both the Alt a 1 protein and wildtype spores stimulated the production of CXCL1, IL-4, and IL-13 in mice following intranasal delivery of spores or Alta1 protein. Infiltration of effector cells into the mouse airways was also observed. Alta1 protein and wildtype Alternaria spores evoked the secretion of pro-inflammatory cytokines in treated airway epithelial cells. In contrast, spores of the *alta1* deletion mutant, or an Alta1 mutant protein lacking enzymatic activity, were unable to induce cytokine production compared to wildtype spores suggesting that this single protein may play a major role in inducing innate immune responses in airway epithelium. Finally, we have discovered that Alta1 rapidly enters human airway cells. Results will be discussed.

563. Comparative analysis of the plant responses to the host-selective toxins Ptr ToxA and Ptr ToxB. I. Pandelova, M. Figueroa, L. Wilhelm, A. Mankaney, T. Mockler, L. Ciuffetti. Oregon State University, Corvallis, OR

Host-selective toxins (HSTs) are pathogen produced molecules that when infiltrated into specific host genotypes reproduce the symptoms triggered by the pathogen. *Pyrenophora tritici-repentis* (Ptr), a necrotrophic fungus and the causal agent of tan spot of wheat, produces one or a combination of HSTs necessary to colonize the plant. The resulting susceptibility phenotype depends on the nature of the HST(s) that is being produced. Necrotic symptoms caused by Ptr can be attributed to the production of Ptr ToxA (ToxA), while chlorotic symptoms are caused by Ptr ToxB (ToxB). In this study we performed a comparative analysis between ToxA- and ToxB-induced transcriptional and biochemical responses of the sensitive cultivar Katepwa. These analyses established that the later onset of symptom development in ToxB-treated leaves correlated with a delayed molecular response to the toxin. Both ToxA and ToxB treatments trigger defense responses, e.g., up-regulation of WRKY transcription factors, PR genes, and genes associated with the phenylpropanoid and jasmonic acid pathways, and ROS accumulation. Therefore, ToxA and ToxB act as elicitors by evoking defense responses similar to those associated with plant resistance. Biochemical evidence also shows that ethylene biosynthesis is involved in acceleration of cell death in ToxA-treated leaves only. The results of this study further support the hypothesis that plant defense responses are exploited by necrotrophic fungi in order to induce cell death.

564. Metabolic remodeling alters virulence in *Cryptococcus neoformans.* Emma Griffiths[1], Louis de Repentigny[2] and Jim Kronstad[1]. [1]Michael Smith Laboratories, British Columbia, Canada [2]Universite de Montreal, Montreal, Canada griffie@msl.ubc.ca

Cryptococcus neoformans causes life-threatening pulmonary infections and meningitis in immunocompromised individuals. If left untreated, cryptococcosis is 100% fatal and ~650,000 deaths occur globally from the disease each year. Existing antifungals suppress, but do not eradicate, infection and prolonged use is associated with resistance and host toxicity. To design more efficacious therapeutics, it is crucial to understand the pathogenic adaptations of *C. neoformans* that contribute to fungal proliferation and disease in humans. Sufficient acetyl-CoA pools are required for a number of critical cellular processes. Transcriptional analysis of *C. neoformans* in a mouse lung model revealed an upregulation of genes involved in acetyl-CoA synthesis and utilization, suggesting specific metabolic changes during infection. Studies suggest that acetate/acetyl-CoA play important roles in glucose regeneration and other important metabolic changes during infection in the nutrient deficient environment of the phagosome. We hypothesized that mutants deficient in acetyl-CoA metabolism will exhibit attenuated virulence and defective growth characteristics. While the deletion of certain acetate/acetyl-CoA-related transport and metabolic genes did not exhibit growth defects or altered virulence factor expression, the deletion of ATP citrate lyase (*acly*) resulted in growth, virulence and mating defects, as well as increased susceptibility to fluconazole. These results indicate that different metabolic genes have specific effects in elaborating virulence in *C. neoformans*, and that these critical pathways, when inhibited, cannot be compensated for by other processes.

565. The Rim101 transcription factor regulates capsule, iron, and virulence in *C. neoformans.* Teresa O'Meara, Fred Dietrich, and J. Andrew Alspaugh. University Program in Genetics and Genomics, Duke University, Durham NC

In order for *Cryptococcus neoformans* to cause disease, it must be able to respond to the hostile environment of a human host. Towards this end, *C. neoformans* integrates signals from two conserved signaling cascades, the cAMP/PKA and RIM pathways, to activate the Rim101 transcription factor. We recently demonstrated that PKA phosphorylation is required for Rim101 truncation and localization to the nucleus. New deep RNA sequencing data provides evidence that Rim101 shares extensive downstream targets with PKA, further demonstrating the novel interaction between CnRim101 and PKA. When examining the role of Rim101 in mouse models of cryptococcosis, we discovered that the *rim101* mutant strain was hypervirulent and that the disease progression in mice varied significantly from wild-type infections. This was unexpected, as the *rim101* mutant has defects in growth in low iron medium, which is a host-relevant condition, and a defect in capsule attachment, which is an important virulence factor. However, the data support the hypothesis that the *rim101* mutant strain is better able to grow under acid conditions, and therefore able to proliferate within the acidic phagolysosome of macrophages. Additionally, deep sequencing, bioinformatic analysis, and gel shift assays revealed the role of Rim101 on the transcription of many iron- regulating genes, including Cir1 and HapX.

566. Beta-oxidation of fatty acids, a common virulence factor of human and plant fungal pathogens. Matthias Kretschmer, Jana Klose, Joyce Wang and Jim Kronstad UBC, Michael Smith Laboratories, 2185 East Mall, Vancouver, British Columbia, Canada, V6T1Z4; e-mail: kronstad@msl.ubc.ca

Cryptococcus neoformans causes infections in immunocompromised people. *Ustilago maydis* is used to study biotrophic interactions of plants and fungal pathogens. Both fungi belong to the Basidiomycota and show yeast-like morphology in culture. After mating and a switch from yeast to filamentous growth, *U. maydis* can establish an infection of corn plants. Fatty acids can also trigger this morphological transition. SAGE data showed upregulation of beta-oxidation genes during animal infections of *C. neoformans*. Fatty acids are essential components of plasma membranes, are signaling molecules and are a major energy storage. Potentially fatty acids of the host could be used by pathogens to establish an infection, when glucose availability is limited. To investigate the importance of beta-oxidation for pathogenesis, essential enzymes for the peroxisomal (mfe2/mfe2b) and the mitochondrial (had1/had2) beta-oxidation pathway were deleted in both fungi The mutants showed reduced growth on different fatty acids (C4-C18) as sole carbon sources and in the case of *U. maydis* also the transition to filamentous growth was compromised. Deletion of mfe2/mfe2b resulted in reduced virulence of *C. neoformans* and *U. maydis*. The deletion of had1/had2 in *U. maydis* also resulted in reduced virulence. This study clarifies fungal beta-oxidation pathways and shows a connection of beta-oxidation of fatty acids and virulence/pathogenicity of human and plant fungal pathogens.

567. Whole genome analysis of *Magnaporthe oryzae* secreted proteins during pathogenesis. Xiaoyan Gong[1], Mihwa Yi[2], Junhuan Xu[1], Baohua Wang[1], Oscar Hurtado[1], Congqing Wu[1], Barbara Valent[2] and Mark Farman[1] [1]Dept. of Plant Pathology, University of Kentucky, Lexington, KY USA; [2]Dept. of Plant Pathology, Kansas State University, Manhattan, KS, USA

Plant pathogenic fungi have the capacity to secrete many proteins with diverse roles in plant-microbe interactions. However, information on the temporal and spatial patterns of protein secretion during plant infection is extremely limited. To address this gap in understanding, we are performing high throughput localization of *Magnaporthe oryzae* secreted proteins during penetration of, and growth inside, rice leaf sheaths. Bioinformatic analysis of the *M.oryzae* genome identified over 900 genes coding for proteins that are predicted to be secreted outside of the fungus. Using newly developed Gateway vectors, we have generated more than 600 secreted protein::green/red fluorescent protein (GFP/RFP) fusion constructs. These have been introduced into *M.oryzae* via Agrobacterium-mediated transformation and transformants were inoculated onto rice leaf sheaths. Live cell imaging was used to detect the proteins in spores, appressoria, during biotrophic growth and, in some cases, during lesion development. To date, we have tested over 600 proteins, approximately 50% of which are expressed in spores or at some time during pathogenesis. At least 15 distinct localization patterns have been identified and examples will be presented.

https://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

568. Genomics of Alternaria-Mammalian Host Interactions. Christopher B. Lawrence Virginia Bioinformatics Institute, Blacksburg, VA 24061

Sensitization and exposure to the airborne fungus Alternaria increases the chances of developing allergic rhinitis, CRS, asthma or exacerbates preexisting asthmatic conditions. Our laboratory is focused on taking a genomics level approach in identifying the proinflammatory/immunomodulatory proteins produced by this clnically relevant fungus. In this regard we have sequenced and annotated several Alternaria genomes using Sanger methods and the Roche 454 titanium sequencing platform. RNA-seq has been performed with Illumina technology to identify genes expressed specifically during spore germination in the presence of human airway epithelial cells. We have also performed Affymetrix gene chip experiments to profile the human airway epithelial cell response to germinating fungal spores. In addition to in vitro studies with human and mouse cells, mouse models of fungal exposure to profile innate and adaptive immune responses to Alternaria spores, antigen preparations, and proteins have been developed. During these studies we have identified several immunomodulatory Alternaria proteins including the major allergen Alta1. We have also shown that Alternaria evokes a potent innate/adaptive immune response including Th2 cytokines/chemokines, and TLR-dependent and IFN-g signaling pathways. Results of these collective studies will be presented.

569. Tracking the Secreted Proteins of the Rice Blast Fungus during Early Biotrophic Invasion of Rice. Mihwa Yi[1], Oscar Hurtado[2], Mark Farman[2], and Barbara Valent[1] [1]Kansas State University, Manhattan, Kansas, USA [2]University of Kentucky, Lexington, Kentucky, USA

The fungus *Magnaporthe oryzae* is the causal agent of the devastating rice blast disease and it is threatening stable rice production worldwide. Despite extensive research efforts, the repertoire of secreted proteins that are involved and how the fungus is coping with plant responses during the early infection *in planta* are largely unknown. We targeted fungal secreted protein candidates that were highly upregulated (>10-fold) during the early biotrophic infection stage compared with the vegetative growth stage. We labeled 134 proteins with C-terminal mRFP using a high throughput Gateway cloning system and tracked the fusion proteins *in planta* using the rice sheath assay. We found that 80 fluorescently labeled proteins, encoded by mostly unique genes in the *M. oryzae* genome (81%), were specifically expressed and localized in various patterns associated with the Biotrophic Interfacial Complex (BIC). In addition, 25 of the BIC-localized proteins were detected in the cytoplasm of invaded rice cells, and 22 of these translocated proteins moved in advance into uninvaded cells. Three proteins accumulated at the cell wall locations where the fungus crossed into neighboring cells, possibly playing roles as the invasive hyphae move across the host cell wall. These findings provide initial steps for the identification of novel blast effector proteins and better understanding of the early infection mechanism *in planta*.

570. Biomass conversion in the fungal garden of the leaf-cutter ant *Acromyrmex echinatior*. Morten N. Grell[1*], Sanne Nygaard[2], Tore Linde[1,2], Jacobus J. Boomsma[2], and Lene Lange[1**]. [1]Section for Sustainable Biotechnology, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Copenhagen, Denmark. [2]Center for Social Evolution, Department of Biology, University of Copenhagen, Denmark. [*]mng@bio.aau.dk [**]lla@adm.aau.dk

It has been demonstrated that fungal enzymes play a significant role in the fungal garden conversion of the fresh-cut leaves into accessible food for the ant larvae (Schiøtt et al. 2008, BMC Microbiol, 8:40; Licht et al. 2010, Evolution 64: 2055-2069). However, so far specific documentation of conversion of also the cellulose fibers itself has been scarce. In the current study, we have taken an experimental approach allowing us to discover which genes are specifically expressed in the upper, middle, and bottom layers of the fungal garden. Using the DeepSAGE technique (Nielsen et al. 2006, Nucleic Acids Res 34:e133) short cDNA tags of the mRNA molecules produced in each of the fungal garden layers were achieved. Subtractive comparisons were made, identifying the genes at least 2x over-expressed in the bottom layer compared to the upper layer. Extended sequence of the selected genes were acquired through matching to an EST library produced from the same fungal garden material and 454 genome sequencing data of the fungal symbiont *Leucoagaricus gongylophorus*. Based on Blast searches, the deducted function of the identified genes was achieved. The results provided interesting new knowledge to understand biomass degradation in the leaf-cutter ant fungal garden: We found the full spectrum of cellulose degrading enzymes among the over-expressed genes in the bottom layer of the fungal garden: Glycoside hydrolases of families GH3, GH5, GH6, GH7, and GH61, and additional proteins containing carbohydrate-binding module CBM1. In conclusion, also the cellulose fibers of the ant assembled leaf cuts are degraded by fungal enzymes. The degradation appears to take place primarily in the bottom layer of the fungal garden.

571. The *Sclerotinia sclerotiorum* Cu,Zn Superoxide Dismutase (SsSOD1) is Essential for Pathogenicity and Protection against Oxidative Stress. Selvakumar Veluchamy and Martin B. Dickman (mbdickman@tamu.edu; sveluchamy@ag.tamu.edu) Institute for Plant Genomics and Biotechnology, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic fungal pathogen with a broad host range that produces the non-specific phytotoxin and key pathogenicity factor, oxalic acid (OA). One of the earliest defense responses against pathogens is the accumulation of reactive oxygen species (ROS) in plants. The superoxide ion is a key intermediate in the generation of ROS and is receiving increasing attention in fungi and in regulation of plant-microbe interactions. Superoxide dismutases (SOD) participate in frontline defense and can detoxify reactive superoxide radical anions to hydrogen peroxide and thus may be important in regulation of fungal infection. We have identified an *S. sclerotiorum* SOD (*SsSOD1*) with high similarity to CuZn SODs. SsSOD1 was significantly induced during Sclerotinia interaction with plant hosts or following treatment with oxidants. Fungal treatment with CuZn SOD inhibitors; diethyldithiocarbamate (DETC) and triethylenetetramine (TETA) resulted in delayed hyphal growth and sclerotial development in a dose-dependent manner. Mutants carrying an *SsSOD1* deletion (*SsSOD1*) exhibited morphological defects to those observed with the CuZn SOD inhibitor treatment. Moreover, mutant was more sensitive than wild-type to menadione, a redox cycling agent. This mutant was also significantly less pathogenic on both tomato and tobacco plants. Furthermore, as evidenced by strong NBT staining, greater accumulation of superoxide was observed in leaves following mutant inoculation. Conversely, upon wild-type inoculation, a cleared stain free area was evident around the infection point. Taken together, these results suggest that *S. sclerotiorum*, via SsSOD1, is able to effectively suppress the host oxidative response to achieve pathogenic success.

572. 400 Million Year Old Facultative Dependence of Arbuscular Mycorrhizal Fungi on Glomeribacter Endobacteria. Stephen J. Mondo[1], Kevin H. Toomer[1], Joseph B. Morton[2], and Teresa E. Pawlowska[1] [1]Department of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY 14853-5904; [2]Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506-6108

Evolutionary theory predicts that over long periods of time, reciprocal selection in mutualistic endosymbioses will lead to increased symbiont interdependence and strict vertical transmission of endosymbionts. We tested this prediction in the mutualistic symbiosis between arbuscular mycorrhizal fungi (Glomeromycota) and *Ca.* Glomeribacter gigasporarum endobacteria. We generated multilocus datasets for both symbionts to examine their populations for evidence of cospeciation and recombination. We surveyed 115 isolates from 34 experimental fungal populations representing the diversity of the Gigasporaceae family. We found that even within closely related fungal groups, endobacterial presence varied, indicating that endobacteria are not essential for the survival of their hosts. However, despite being facultatively associated, we detected significant evidence for cospeciation between symbionts. This global pattern of cospeciation is largely the result of several significant contributions from relatively few lineages and weaker or no contributions from most others. Host switching and recombination amongst endosymbionts are the factors responsible for the absence of cospeciation in non-cospeciating host-endosymbiont pairs. As cospeciation implies simultaneous speciation of host and symbiont, we used the host fossil record to infer that the association of AM fungi with Glomeribacter is at least 400 million years old. Unlike most essential, obligate endosymbioses that have rapidly evolved from facultative interactions, the association between Glomeromycota and Glomeribacter appears to be permanently locked in a facultative state despite its ancient origin.

573. SCAT1, A Novel *Sclerotinia sclerotiorum* Catalase, Plays a Role in Cell Wall Integrity Maintenance and Ergosterol Pathways. Selvakumar Veluchamy and Martin B. Dickman (mbdickman@tamu.edu; sveluchamy@ag.tamu.edu) Institute for Plant Genomics and Biotechnology, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843

Catalases are antioxidant enzymes that convert hydrogen peroxide (H_2O_2) to water and molecular oxygen, and play an important role in the detoxification of reactive oxygen species. Fungi typically carry several catalases that perform diverse cellular functions. The genome of the necrotrophic fungal pathogen *Sclerotinia sclerotiorum* contains seven predicted catalase genes; three peroxisomal catalases, two catalases (cell-wall associated), one catalase-peroxidase, and one catalase A. The catalase A gene, designated "SCAT1" specifically induced following fungal inoculation onto tomato. SCAT1 encodes a protein containing a glutamine amidotransferase (GATase1)–like domain with an unknown function. A deletion mutant (ÄScat1) was generated and transformants displayed growth retardation and delayed sclerotia formation in culture. The wild-type phenotype was recovered by reintroducing *SCAT1* into the *ÄScat1* mutant background. Surprisingly, mutant showed accelerated growth on agar plates containing 5 to 20 mM H_2O_2. In the light of these results, mutant was sensitive to sodium dodecyl sulfate (SDS) but not to the cell wall modifiers 1,3-beta glucan synthase inhibitor caspofungin (antifungal compound that block cell wall synthesis) or the chitin-binding dye Calcofluor White. Mutant was also hypersensitive to osmotic stress such as sorbitol and NaCl, suggesting that *SCAT1* might function in cell wall integrity. However, ÄScat1 was more resistant to Amphotericin B and Nystatin than the wild-type. Amphotericin B associates with ergosterol, the principal component of fungal cell membranes and is involved in the maintenance of ion leakage. Interestingly the *ÄScat1* was highly sensitive to terbinafine, a potent inhibitor of the ergosterol biosynthetic enzyme squalene epoxidase in fungi. Our results show that *SCAT1* is involved in cell wall strengthening and ergosterol pathways.

574. Comparison of strain genotype, phenotype and patient clinical reaction to the opportunistic fungal pathogen *Cryptococcus neoformans*. Oleksandr Moskalenko, Darin Wiesner, David Boulware and Kirsten Nielsen. University of Minnesota, Minneapolis, MN 55455.

Cryptococcus neoformans is an opportunistic human fungal pathogen that afflicts immunocompromised individuals such as HIV/AIDS patients. In sub-Saharan Africa, cryptococcal meningitis is frequently the first and most fatal AIDS- related illness with up to 30% infection rate and 70% annual mortality totaling more than 504,000 deaths in 2008. Worldwide numbers of over a million cryptococcal infections reflect the widespread distribution of this pathogen. As the epidemiological scale of the *Cryptococcus* infections grows, so does the importance of improving clinical treatments. One possible approach to improving clinical outcomes while preserving the patient quality of life is to treat the specific combination of pathogen virulence and host response in each clinical case. We have isolated 124 *C. neoformans* strains from AIDS patients suffering from cryptococcal meningitis in the Kampala region of Uganda in sub- Saharan Africa. Strain genotypes were determined by the MLST approach. We performed analyses of the genotypes, phenotypic virulence factors, patient clinical presentation and immunological response. Comparison of strain genotypes with phenotypic characteristics and patient clinical presentation revealed relationships that suggest genotype may be clinically informative.

575. Proteomic analysis of Cryptococcus neoformans to identify binding partners and substrates of Pka1 associated with virulence. Geddes, J.M.H., Choi, J., Cadieux, B., Foster L.J., Kronstad J.W.

Cryptococcus neoformans is an opportunistic, yeast-like fungus with worldwide distribution. The pathogen is capable of infecting immunocompromised individuals to cause life-threatening cryptococcal meningitis. The ability of C. neoformans to cause disease depends on three virulence factors: the polysaccharide capsule, the ability to grow at 37oC, and melanin production. Capsule and melanin production are regulated by the cAMP/Protein Kinase A (PKA) signaling pathway, and part of this regulation occurs via control of expression of secretory pathway components. We hypothesize that PKA phosphorylates proteins associated with the secretory pathway to regulate capsule production, the extracellular proteome, and cryptococcal virulence. To identify potential binding partners and substrates for Pka1 in C. neoformans, an immunoprecipitation assay was performed between purified Pka1 and protein extracts of a C. neoformans wild-type strain and a pkr1 mutant. Previously, several PKA phosphorylation targets were identified in C. neoformans, including transcription factors Ste12 and Ngr1, and the exocyst protein Sec15. Bioinformatic studies of the C. neoformans proteome identified 1619 predicted Pka1 targets with the recognition sequence: R-X-X-R-X-X-S/T. Overall, this work will identify protein-protein interactions between Pka1 and its targets in C. neoformans, leading to further characterization of the role of Pka1 in virulence, and regulation of the extracellular proteome.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

576. *RIG1*, a gene essential for pathogenicity in *Magnaporthe oryzae*, is representative of Gti1_Pac2 family members required for invasive growth in fungal pathogens of plants and animals. Amritha S. Wickramage, M. Alejandra Mandel and Marc J. Orbach, Department of Plant Sciences, Division of Plant Pathology and Microbiology, University of Arizona, Tucson, AZ 85721.

Magnaporthe oryzae has two members of the fungal gene family Gti1_Pac2 – a group of genes that regulate phase transition in human pathogens *Candida albicans (WOR1), Histoplasma capsulatum (RYP1)* and the plant pathogen *Fusarium oxysporum (SGE1)*. Deletion mutants generated separately for each gene showed that one member - *RIG1 (Required for Infectious Growth 1)* - but not the other, MGG_06564, was important for pathogenicity. RIG1 is dispensable for vegetative growth, but *RIG1* deletion mutants (*rig1*) are non- pathogenic, even after removal of the penetration barrier. Microscopic analysis of the mutant from germination through infection indicate that the mutant forms significantly longer germ tubes than the wildtype parental strain 70-15, but forms appressoria that are morphologically and functionally identical to those of 70-15. Observation of fluorescent protein- tagged strains indicates that the mutant fails to form primary infectious hyphae *in planta*: the point synonymous to phase transition in animal pathogenic fungi. RIG1 transcript levels are upregulated in mature appressoria of the wild type, relative to the mycelium. Based on the observed function of *RIG1* homologs in the Gti1_Pac2 family, we propose that these members represent a consensus gene required for invasive growth within the host in both animal- and plant-pathogenic fungi.

577. A systems approach to regulation of a fungal virulence factor. Haynes, Brian C.[1], Skowyra, Michael L.[2], Gish, Stacey R.[2], Williams, Matthew[2], Brent, Michael R.[1],and Tamara L. Doering[2]. [1]Department of Computer Science, Washington University School of Engineering, St. Louis, MO, USA. [2] Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA.

Cryptococcus neoformans is a pathogenic yeast responsible for serious opportunistic infections that lead to over 600,000 deaths annually worldwide. The major virulence factor of this fungus is a polysaccharide capsule, which is dramatically regulated by growth conditions. Elegant work by several groups working in this field has identified a handful of transcription factors that impact capsule phenotype. However, a more global picture of the relationships between these factors is lacking. We have combined transcriptional and phenotypic profiling to model the capsule regulatory network in *C. neoformans*. This approach has implicated an array of putative transcription factors in capsule regulation, and uncovered relationships between several of these and known regulators. Deletion of the gene encoding one putative transcription factor, Bch1p, yields cells with a notable reduction in capsule size and loss of virulence in animal models. Progress on this project and this rapidly developing area will be presented. This work is supported by NIH grant GM071007.

578. Necrotrophic, host-generalist species in the Sclerotiniaceae are characterized by early, abundant oxalic acid production during plant infection. Marion Andrew[1] and Linda M. Kohn[1]. [1]University of Toronto, Department of Ecology and Evolutionary Biology, Mississauga, ON, Canada.

Given a set of pathogenicity-related genes among a set of species, why is one species a biotroph and specialist while another is a necrotroph and generalist? Is it the result of selection on primary sequence, or on proteins, or alternatively, differences in the timing and magnitude of gene expression? The Sclerotiniaceae (Leotiomycetes, Helotiales) are a strongly supported and relatively recently evolved clade that includes biotrophs, necrotrophs, host generalists and host specialists. We compared 9 species in 4 genera (*Sclerotinia*, *Botrytis*, *Myriosclerotinia* and *Monilinia*). Our candidate genes code for pathogenicity-related proteins involved in cell wall degradation and the oxalic acid pathway. Rate- and site-specific likelihood analyses provided evidence for positive selection acting on 5 of the 8 pathogenicity-related genes, consistent with the prediction of the evolutionary arms race model. *In planta* experiments were performed by inoculating whole plants of *Arabidopsis thaliana* with mycelial plugs of a panel of isolates representing host generalists and host specialists, and the spectrum of trophic types in the Sclerotiniaceae. Expression levels of the oxalic acid pathway-related genes, *oah* and *pac1*, were measured using qRT-PCR at 0, 1, 2, 4, 8, 12, 24, 48 and 72 hours post-inoculation. The results indicated that the magnitude and timing of expression of the *oah* gene differs between the necrotrophs/host generalists, and those species that were host specialists and/or biotrophs. These results support the hypothesis that early, abundant oxalic acid production is important for infection among the necrotrophic/generalist species in the Sclerotiniaceae, but not in the biotrophs or specialists.

579. Effector mining in the genome of the Dothideomycete plant pathogen, *Pyrenophora tritici-repentis* – insights from resequencing multiple isolates. V. Manning [1], L. Wilhelm [1], I. Pandelova [1], M. Figueroa [1], W. Holman [1], I. Grigoriev [2], L. Ma [3]& L. Ciuffetti [1] [1]Oregon State University, Corvallis, OR, 97331. [2] JGI, Walnut Creek, CA, 94598. [3] Broad Institute/MIT, Cambridge, MA, 02141.

Tan spot of wheat is the result of infection of susceptible wheat genotypes with isolates of *P. tritici-repentis* (Ptr) that produce a variety of effectors, including host-selective toxins (HST). HSTs can be proteins or secondary metabolites. Race 1 isolates of Ptr produce at least two HSTs, the protein Ptr ToxA, and the small molecular weight non-proteinaceous Ptr ToxC; evidence suggests the presence of at least one additional HST. To identify effectors required for disease versus infection in the absence of disease, we utilized genomic sequence data from the Race 1 reference genome and resequenced a pathogenic isolate that produces an alternate HST, Ptr ToxB, and a non-pathogenic isolate. Comparative analyses show that resequenced isolates are missing a 135 kb region that harbors the *ToxA* gene and others that may contribute to pathogenicity. Furthermore, several putative secreted proteins, which may represent additional pathogenicity factors, are differentially represented in the three isolates. All putative polyketide synthases and non- ribosomal peptide synthetases that may contribute to secondary metabolite production were identified in the reference genome and their presence/absence in the other isolates determined. This comparison may facilitate identification of the biosynthetic pathway necessary for ToxC production. Comparison of sequences shared by the reference genome and the re-sequenced genomes revealed a greater number of SNPs in the non-pathogen. Interestingly, Ptr possesses a Histone deacetylase biosynthetic cluster closely related to the apicidin cluster of *F. incarnatum* and may represent a previously unknown virulence factor in the Ptr-wheat pathosystem.

580. Mechanisms of delivery of rice blast effector proteins into live rice cells. Chang Hyun Khang and Barbara Valent Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506 ckhang@ksu.edu; bvalent@ksu.edu

Magnaporthe oryzae is a hemibiotrophic pathogen causing blast disease on rice and wheat. During biotrophic invasion, *M. oryzae* delivers effector proteins into living host cells to manipulate host responses for successful infection. Some biotrophy-associated secreted (BAS) proteins, including known effectors such as PWL2, accumulate in a highly localized structure, the Biotrophic Interfacial Complex (BIC), and they are translocated into the rice cytoplasm. In contrast, the BAS4 protein is uniformly retained within the extrainvasive hyphal membrane (EIHM) that surrounds the fungus inside rice cells, and it fails to enter the host cytoplasm. We have determined that the upstream sequence (promoter and/or 5'UTR) of BAS genes plays a critical role in determining BAS protein localization. The expression of the PWL2 protein with the BAS4 upstream sequence resulted in a BAS4-like localization pattern and impaired host translocation. Expression of the BAS4 protein with the PWL2 upstream sequence resulted in modified BIC localization without uniform accumulation surrounding the hyphae. The PWL2 upstream sequence and N-terminal signal peptide- coding sequence were sufficient for BIC targeting and host translocation, suggesting the PWL2 mature protein does not contain a required motif. However, the PWL2 mature protein or RNA sequence is required for efficient secretion to the BIC and host translocation. Further characterization of the PWL2 upstream sequence and signal peptide sequence will be presented.

581. Cloning of the avirulence gene *AvrPm3* in *Blumeria graminis* f. sp. *tritici.* Francis Parlange, Simone Oberhaensli, Thomas Wicker, Tina Jordan, Lisa Haldemann, Beat Keller University of Zurich, Institute of Plant Biology, Zurich, Switzerland f.parlange@access.uzh.ch

Wheat powdery mildew is caused by the obligate biotrophic ascomycete *Blumeria graminis* f.sp. *tritici* (*Bgt*). Interaction is determined by the gene-for-gene model. The wheat resistance gene *Pm3* was cloned in our group and shown to confer specific resistance to *Bgt* races in an allelic manner. Our objective is to clone the fungal avirulence gene specifying the *Pm3*-mediated resistance, *AvrPm3*. This would allow elucidation of the molecular mechanisms of recognition by Pm3 and of induction of defence processes. It would also provide information on co-evolution between both partners of the interaction, and help the characterization of additional AVR or effectors in the *Bgt* genome. Using a positional cloning strategy, *AvrPm3* was localized in a repeat-rich (63%) genomic region of 30 kb. No homology was observed with sequences in the databases. Candidate sequences identified using gene-prediction programs were investigated for expression, for polymorphisms associated with the phenotype in natural isolates, and for presence of signal peptide. One particularly interesting sequence is now under functional validation process through transient assay in wheat. We are also developing additional approaches for validation, such as transient assay in *Arabidopsis thaliana* and yeast-two-hybrid experiment, and continue investigations on the other candidate sequences.

582. Examining the Molecular Basis of Pathogenesis of *Batrachochytrium dendrobatidis*: A Functional Genomics Approach. Thomas J. Poorten[1] and Erica Bree Rosenblum[1,2]. [1]Bioinformatics and Computational Biology, University of Idaho, Moscow, Idaho. [2]Biological Sciences, University of Idaho, Moscow, Idaho. Corresponding author: tpoorten@uidaho.edu

The fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), has been implicated as a cause of amphibian declines worldwide. Bd is a broad host range pathogen that has recently emerged and rapidly spread to every continent containing amphibians. However the determinants of its pathogenicity have remained a mystery. Our study seeks to gain insights into the molecular basis of Bd pathogenicity. We examined the genome-wide gene expression profile of Bd during growth on two substrates: standard Bd culture media (1% tryptone) and dissected, sterilized frog skin. The experiment was performed using a gene expression microarray customized to detect 13,891 Bd transcripts found in the Bd genome. Analysis of these data showed that nearly half of the transcripts were differentially expressed when comparing growth on frog skin to culture media. A thorough examination of differentially expressed transcripts revealed the potential importance of 3 protease gene families for pathogenicity as evidenced by elevated gene expression during growth on frog skin. The application of this new functional genomics technology to a non-model organism allows us to rapidly gain insights into the nature of pathogenicity of this recently emerged pathogen.

583. Metabolomics meets Genomics: Solving the puzzle of how multiple cyclic oligopeptides are synthesised by epichloae endophytes via a single ribosomally encoded gene, *gigA*. Linda J Johnson, Albert Koulman, Geoffrey Lane, Karl Fraser, Christine Voisey, Jennifer Pratt, Gregory Bryan and Richard D Johnson. AgResearch, Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand. Phone: +64 6 351 8090. Fax: 15 +64 6 351 8032. Email: richard.johnson@agresearch.co.nz

Epichloae endophytes live symptomlessly within the intercellular spaces of cool-season grasses, and confer a number of biotic and abiotic advantages to their hosts. Metabolomics analysis, by direct infusion mass spectrometry, of the *Epichloë festucae*-perennial ryegrass association identified a new class of endophyte derived compounds (multiple cyclic oligopeptides) only in the guttation fluid of infected plants. In a parallel programme to knock out the most highly expressed endophyte gene during symbiosis, *gigA*, we found that all cyclic oligopeptides (COPs) were eliminated in the delta *gigA* mutant. Initial analysis of the predicted GigA protein, however, suggested that COPs were not a direct product of the gene. Here we describe how multiple COPs are synthesised via a single ribosomally encoded gene, *gigA*, which is expressed preferentially *in planta* and is one of the most abundantly expressed fungal transcripts in endophyte infected grasses. The GigA protein contains an N-terminal signal sequence and imperfect 27 amino acid repeats which we propose are processed by a kexin protease to yield multiple COPs of 8 or 9 amino acids. Deletion of *gigA*, and re-introduction of the mutant into the host plant, leads to complete loss of COP production, altered hyphal ultrastructure and an increase in fungal biomass. Recent evidence suggests that *gigA* forms part of a gene cluster with a kexin protease and two hypothetical proteins. This is the first report of multiple cyclic peptides being ribosomally encoded from a single gene and we are interested in both the mechanism of cyclisation and the function of these COPs in endophyte-grass symbioses.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

584. Expression and localization of *Magnaporthe oryzae* cell wall degrading enzymes during pathogenesis. Junhuan Xu, Baohua Wang, Mark Farman. Dept. Plant Pathology, University of Kentucky, Lexington, KY USA

Phytopathogenic fungi have the capacity to produce a diverse array of cell wall degrading enzymes (CWDEs) that are predicted to have roles in plant pathogenesis. However, for the vast majority of enzymes it is not known if they are expressed at all within host tissues. To gain a comprehensive understanding of CWDE expression during plant infection, we are using live-cell imaging of RFP/GFP protein fusions to study the entire CWDE complement of *Magnaporthe oryzae*. Preliminary analysis of a selection of enzymes revealed that, as expected, many were present in appressoria. However, very few were expressed in appressoria developing on artificial surfaces - pointing to a role for plant-derived signals in penetration-related CWDE expression. Surprisingly, many of the enzymes were already present in ungerminated spores. None of the CWDEs studied were detected in biotrophic hyphae growing within rice cells but some were still abundantly present in the overlying appressorium, even as late as 48 hours after inoculation, when the fungus had already proliferated extensively within the host. Although all of the CWDEs were repressed during biotrophic growth, many were re-activated and expressed very highly at the onset of lesion formation, approximately 96 to 120 hours after inoculation. In general, our data support the predicted roles for CWDEs during penetration and lesion formation but indicate that they are not expressed during proliferative growth *in planta*.

585. Genetic and cytological analysis of *Magnaporthe oryzae* "non-host" interactions. Melanie Heist, David Thornbury, Mark Farman Dept. Plant Pathology, University of Kentucky, Lexington, KY USA

Magnaporthe oryzae isolates from perennial ryegrass are unable to infect rice due to the elicitation of a strong, "non-host" type resistance response. We are using cytological studies of infection sites in concert with classical genetics to elucidate the genetic control of this non-host interaction. The laboratory strain 2539 was crossed with the prg pathogen FH with the goal of introducing fertility. Progeny from this cross exhibited a segregation ratio of 15 avirulent :1 virulent on rice cultivar 51583. This indicated the assortment of four unlinked avirulence genes – one from 2539 and three from FH. Selected avirulent F1 progeny were then crossed to the rice pathogens Guy11 and ML33. Avirulence:virulence ratios for three populations indicated the segregation of two to three avirulence genes. Interestingly, inoculation of rice cultivars YT-16 and M202 revealed two additional AVR genes that were not recognized by 51583. Backcrosses with Guy11 are being performed to segregate the individual AVR genes away from one another. Cytological studies showed that strain FH was unable to grow inside rice cells, whereas 2539 grew within the first invaded cell before triggering the host's defenses. Most progeny isolates grew to varying degrees within the first host cell, while some even moved into adjacent cells before the host defense response stopped growth. Together our data suggest that the strong, non-host resistance to *M. oryzae* is a cumulative response resulting from multiple AVR protein:resistance protein interactions.

586. Severe symptoms observed for infected RNA silencing mutants of *Cryphonectria parasitica* are associated with a central region of the Hypovirus genome. Xuemin Zhang, Diane Shi and Donald Nuss Institute for Bioscience and Biotechnology Research, University of Maryland 9600 Gudelsky Dr, Rockville, MD 20850 dnuss@umd.edu

Hypovirus infection of the chestnut blight fungus *Cryphonectria parasitica* attenuates virulence on chestnut trees and induces a wide range of phenotypic changes. Upon virus infection, the RNA silencing pathway of *C. parasitica* is induced as an antiviral defense response. Disruption of the *C. parasitica* RNA silencing antiviral pathway results in very severe symptoms following infection by several mycoviruses, especially the hypovirus type species CHV-1/EP713. In this study, we showed that not all hypoviruses cause severe phenotypic changes of the fungal host in the absence of the RNA silencing pathway. Infection of the *C. parasitica dcl-2* RNA silencing mutant strain with hypovirus CHV- 1/EP721, which is 99% identical to CHV-1/EP713, resulted in symptoms similar to those exhibited by the infected wild-type *C. parasitica* strain. By swapping domains of these two viruses, we mapped the region that is associated with the severe phenotypic change in the *dcl-2* mutant to a 2.5 kb domain located in the central part of the CHV-1/EP713 genome. Chimeric infectious viral cDNA clones carrying this portion of the hypovirus genome caused phenotypic changes in the *dcl-2* mutant similar to that caused by the corresponding parental viruses. The potential for using these chimeric viruses as viral expression vectors is currently being tested.

587. Further characterization of the *MST12* transcription factor gene in *Magnaporthe oryzae.* Guo-Tian Li, Xiao-Ying, Zhou, Sheng-Li, Ding, and Jin-Rong Xu. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907 USA li4@purdue.edu

In *Magnaporthe oryzae*, the *PMK1* MAP kinase regulates appressorium formation and infectious growth after penetration. Homologs of *PMK1* have been shown in other plant pathogenic fungi to be important for various plant infection processes. However, there are only limited studies on transcription factors and genes regulated by this MAP kinase pathway. In *M. oryzae*, one of its downstream transcription factors is *MST12*, the *MST12* homolog of *Saccharomyces cerevisiae*. *MST12* was essential for infectious growth and the *mst12* mutant was non-pathogenic but still formed appressoria. The expression level of *MST12* was higher in mature appressoria than in vegetative hyphae and conidia. In transformants expressing a *MST12*-GFP construct, fluorescence was observed in the nucleus in appressoria. Pmk1 weakly interacted with Mst12 in yeast two-hybrid assays. It also was co-immunoprecipitated with Mst12-3xFLAG fusion proteins. In agreement with these observations, the MAPK- docking region and phosphorylation site were important for the function of MST12. Expression of the $MST12^{\Delta dock}$ and $MST12^{S133A}$ alleles failed to complement the mst12 mutant. In contrast, *MST12* mutant alleles dele of any one of the two zinc finger domains were still partially functional. In addition, we have used microarray analysis to identify genes regulated by *MST12*. Promoters of genes with significant altered expression levels in the *mst12* mutant were analyzed for common regulatory elements. Preliminary data suggest that Mst12 has a binding site similar to that of yeast Ste12. Overexpression of *MST12* in the *pmk1* and *cpkA* mutants and using Life-Act to examine cytoskeleton defects in *mst12* appressoria are in progress and the resulting data will be presented.

588. Identification of Pathogenicity-Related Genes in the Vascular Wilt Fungus *Verticillium dahlia* by *Agrobacterium tumefaciens*-Mediated T-DNA Insertional Mutagenesis. K. Maruthachalam[1], S. J. Klosterman[2], S. Kang[3], R. J. Hayes[2], K. V. Subbarao[1] 1 Dept. of Plant Pathology, University of California-Davis, Salinas, CA. 2 USDA-ARS, Salinas,CA 3 Dept. of Plant Pathology, The Pennsylvania State University, PA.

Verticillium dahliae is the causal agent of vascular wilt in many economically important crops worldwide. Identification of genes that underpin pathogenicity or virulence may suggest targets for alternative control methods for this fungus. In this study, *Agrobacterium tumefaciens*-mediated transformation (ATMT) was applied for insertional mutagenesis of *V. dahliae* conidia. Southern blot analysis indicated that T-DNAs were inserted randomly into the *V. dahliae* genome and that 69% of the transformants were the result of single copy T-DNA insertion. DNA sequences flanking T- DNA insertion were isolated through inverse PCR (iPCR). *V. dahliae* mutants of particular interest included those that had lost the ability to form microsclerotia. Also, based on the virulence assay of 181 transformants, we identified 20 mutant strains of *V. dahliae* that did not cause symptoms on lettuce plants. Among these mutants, T-DNA was inserted in genes encoding an endoglucase I (*VdEg-I*), a hydroxyl-methyl glutary-CoA synthase (*VdHMGS1*), and a major facilitator superfamily (*VdMFS1*). These results suggest that ATMT can effectively be used to identify genes associated with pathogenicity and other functions in *V. dahliae*.

589. Detection of *Geomyces destructans* in environmental samples. Gargas, Andrea[1], Jeffrey M. Lorch[2, 3], Mark T. Banik[4], Jessie Glaeser[4], David S. Blehert [3], Daniel L. Lindner[4]. [1]Symbiology LLC, Middleton, WI, USA. [2]Molecular and Environmental Toxicology Center, University of Wisconsin – Madison, Medical Sciences Center, Madison, WI, USA. [3] US Geological Survey – National Wildlife Health Center, Madison, WI, USA. [4]US Forest Service, Northern Research Station, Center for Forest Mycology Research, Madison, WI, USA.

White-nose syndrome (WNS) was first identified based on the presence of a psychrophilic fungus, now identified as *Geomyces destructans* (Helotiales, Ascomycota), on the muzzles, wings and pinnae of hibernating bats. Since first detected near Albany, New York in 2006 WNS has spread to 11 U.S. states and two Canadian provinces, killing over one million bats. Delimiting pathogenic *G. destructans* from related fungi present in cave sediment and soil samples is our focus. Our results from soil clone libraries generated with *Geomyces*-selective PCR primers suggest the presence of a diverse range of heretofore undescribed taxa within genus *Geomyces*. Identification of *G. destructans* based solely on ITS rDNA sequence is less than optimal and we are seeking improved identifying markers.

590. The HAP complex in *Fusarium verticillioides* is a key regulator of growth, morphogensis, virulence, and secondary metabolism. John Ridenour and Burt Bluhm Department of Plant Pathology, University of Arkansas, Division of Agriculture, Fayetteville, AR 72701, USA For correspondence, E-mail bbluhm@uark.edu

The ascomycete *Fusarium verticillioides* is a ubiquitous pathogen of maize, attacking seedlings, kernels, and stalks. During favorable conditions, *F. verticillioides* can reduce yields, although of greater concern is the contamination of infected kernels with fumonisins, a group of polyketide-derived mycotoxigenic metabolites. Fumonisins are linked to esophageal cancer and neural tube birth defects in humans, as well as acute toxicoses in livestock. Currently, little is known at the molecular level regarding the regulation of pathogenesis or mycotoxigenesis in *F. verticillioides*. The goal of this study was to identify genes and regulatory mechanisms underlying pathogenicity and fumonisin biosynthesis in *F. verticillioides*. Among eukaryotes, the HAP complex is a highly conserved multimeric transcription factor that binds the consensus DNA sequence CCAAT to regulate gene expression. In the present study, orthologs of the HAP complex core components (*HAP2*, *HAP3*, and *HAP5*) were identified in *F. verticillioides* and functionally characterized via targeted disruption. Disruption of *HAP3* in *F. verticillioides* resulted in a highly pleiotropic phenotype, including reduced radial growth and conidiation, increased biosynthesis of bikarverin, a polyketide-derived secondary metabolite, and a drastic reduction in virulence on maize. Additionally, *HAP2* and *HAP5* disruption mutants of *F. verticillioides* were phenotypically indistinguishable from *HAP3* disruption mutants. This study directly implicates the HAP complex in pathogenesis and secondary metabolism in *F. verticillioides*.

591. Light, Rhythms and Pathogenesis of *Cryphonectria parasitica*. Gloricelys Rivera [1] and Angus L. Dawe [1]. [1] Department of Biology, New Mexico State University, Las Cruces, NM

Circadian rhythms are endogenous cellular clocks that control molecular and physiological functions in most organisms. Within the fungi, *Neurospora crassa* is a model system in the field of chronobiology that has helped define an autoregulatory negative feedback loop. Our project have identified putative open reading frames for orthologous proteins in *Cryphonectria parasitica*, a plant pathogen and causative agent of chestnut blight. We used publicly available *N. crassa* expressed sequences collection to identify 483 putative orthologous genes that are involved in the regulation of the biological clock, within the *C. parasitica* genome. To study light response, a film light spectrum was designed to observed the development of the plant pathogen. Results demonstrated that *C. parasitica* reproduced normally in the presence of blue-light at the same time as decline to develop in red-light. Furthermore, confocal microscopy analysis revealed that *C. parasitica* emits blue-green light under different laser excitation conditions suggesting that the identified orthologous proteins mediate UV and blue-light responses in the fungus. Currently, knockout strains of key regulatory components are being developed to determine if there is a role for circadian rhythms in pathogenicity. The goal of this study is to explore the relationship between circadian rhythms, light response and the potential impact of hypoviruses (virulence attenuating RNA viruses) in plant pathogenesis.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

592. RNA-binding protein mediates *Magnaporthe oryzae* Cellular Differentiation and Plant Infection through Regulation of TOR signaling cascade. Marina Franceschetti [1], Emilio Bueno[1], Richard A. Wilson[2], Grant Calder[1] and Ane Sesma[1] [1] John Innes Centre, Colney lane, Norwich, United Kingdom (ane.sesma@bbsrc.ac.uk) [2] University of Nebraska, Lincoln, USA

The RBP35 protein contains one RNA Recognition Motif (RRM) and six Arg-Gly-Gly tripeptides and is required for *Magnaporthe oryzae* plant invasion. The RRM motif is by far the most common and best characterized of the RNA- binding modules and can function in all post-transcriptional gene-expression processes. RBP35 homologues are found only in filamentous fungi. Western blots identified two RBP35 protein isoforms, the expected full length protein (RBP35a, 44 kDa), and a smaller protein (RBP35b, 31 kDa) that derives from the proteolytic cleavage of RBP35a. Both isoforms bind poly$(G)_{30}$ RNA homopolymers exclusively and show a steady-state nuclear localization. FRAP experiments suggest the ability of RBP35 to form different protein complexes in the nucleus of conidia and appressoria. Truncated and mutated protein variants of RBP35 accumulate in cytoplasmic granules, possibly processing bodies and/or stress granules, indicating an involvement of RBP35 in translational repression of targeted mRNAs. Comparative transcriptome analysis reveal that several signaling pathways are altered in the *rbp35* mutant including the target of rapamycin (TOR), a conserved Ser/Thr kinase that regulates cell growth and metabolism in response to environmental cues. We have optimized tandem affinity purification experiments in order to identify RBP35-interacting proteins and direct mRNA targets. Results of the pull-down experiments using RBP35-HA-FLAG protein fusions will be presented.

593. A molecular insight into algal-oomycete warfare: cDNA analysis of *Ectocarpus siliculosus* infected with the basal oomycete *Eurychasma dicksonii*. Laura J. Grenville-Briggs[1], Claire M.M. Gachon[2], Martina Strittmatter[2], Frithjof C. Küpper[2] and Pieter van West[1]. [1]Aberdeen Oomycete Laboratory, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD, UK. [2] Scottish Association for Marine Science, Scottish Marine Institute, Oban, PA37 1QA, UK.

Oomycetes (or water moulds) are non-photosynthetic Stramenopiles, which exhibit either pathogenic or saprophytic lifestyles. Pathogenic oomycetes infect a remarkable range of hosts including, marine algae, crustaceans, plants, nematodes, fungi, insects, fish and mammals. Many of the most devastating agricultural and aquacultural pathogens belong to the oomycetes. *Eurychasma dicksonii* is an abundant, and probably cosmopolitan, obligate biotrophic pathogen of marine brown algae. Molecular evidence indicates that it belongs to the most basal oomycete clade known so far. It occurs in all cold and temperate seas worldwide. As brown algae make up about 70% of the biomass of temperate and polar rocky shores, we infer that *E. dicksonii* like its terrestrial counterparts, probably shapes natural algal populations, profoundly impacting ecosystem functioning. Here we report the first large scale molecular data acquired on the most basal oomycete to date. 9873 unigenes, totalling over 3.4Mb of sequence data, were produced from Sanger sequenced and pyrosequenced EST libraries, of infected E. siliculosus. 6787 unigenes (69%) are of algal origin, and 3086 (31%) oomycete origin. Here we discuss the physiology of this host-pathogen interaction, in the light of our recent results.

594. Hyphal differentiation in *Magnaporthe oryzae* for plant root colonization. Stephanie Heupel and Natalia Requena Plant Microbial Interaction Dept., Karlsruhe Institute of Technology (KIT) Natalia.requena@kit.edu

Fungal hyphal differentiation during host colonization is an important feature that determines the fate of a fungal-plant interaction. *Magnaporthe oryzae*, the causal agent of rice blast is a well studied leaf pathogen that produces differentiated structures, the appressoria, to penetrate the leaf cuticula. Interestingly, it was shown that *M. oryzae* can also colonize host plants through the roots but that this infection route does not require differentiated melanized appressoria. In contrast, root penetration follows a different morphogenetic program, with the formation of hyphopodia, that is partly shared with other root infecting fungi such as *Fusarium* spp. or arbuscular mycorrhizal fungi. The root colonization pathway is relatively poorly studied and while some components are possibly required for both root and shoot colonization, others are likely to be specific for root infection. In order to identify genetic determinants of root infection that might be eventually conserved with other root infecting fungi, an insertional library of *Agrobacterium tumefaciens*-transformed *M oryzae* has been constructed. Insertional mutants are screened for their ability to infect root and leaves but only mutants able to grow normally on complete media are considered. One of the first identified mutants showed an increased root pathogenicity defect while a reduced pathogenicity on leaves. Phenotypic and genetic analysis of the responsible gene will be presented.

595. Deciphering Effector Function in the *Magnaporthe oryzae* - Rice Interaction. Cécile Ribot[1], Stella Cesari[1], Judith Hirsch[1], Elisabeth Fournier[1], Didier Tharreau[1], Julie Vallet[2], Marc-Henri Lebrun[2], Thomas Kroj[1] [1]BGPI, INRA-CIRAD-SUPAGRO, Montpellier, France; [2]MAP,CNRS-UCB-INSA-BCS, Lyon, France.

Effectors are central to virulence in all pathogenic organisms and to their adaptation to specific hosts. However, the role and molecular function of fungal effector proteins is largely unknown. A better understanding of fungal effectors is necessary for a deeper understanding of fungal pathogenicity and for new perspectives in the development of durably resistant crops. Therefore, we perform systematic and targeted analysis of effectors of the causal agent of blast disease, the ascomycete fungus *Magnaporthe oryzae*, which is the most important rice pathogen and a model for the investigation of the molecular basis of fungal pathogenicity. Genome wide expression analysis allowed us the identification of ~100 effector candidates, secreted specifically during infection. Results from the functional analysis of promising candidates will be presented. Investigation of their localization during infection by life cell imaging studies and in vitro translocation assays indicate their translocation into host cells by unknown mechanisms. In addition, two hybrid screens and over expression studies in rice are beginning to shed light on targeted host proteins and host processes.

596. The *HYR1* gene in the rice blast fungus functions to tolerate plant-produced reactive oxygen species during infection. Kun Huang[1], James Sweigard[2], Jeffrey Caplan[3], Kirk Czymmek[1], Nicole Donofrio[1] [1]University of Delaware, Newark, DE, 19716 [2]DuPont Stine-Haskell, Elkton Rd, Newark, DE, 19711 [3]Delaware Biotechnology Institute, Newark, DE, 19716 ndonof@udel.edu

Plants can mount several types of defense responses to block the pathogen completely or ameliorate the level of disease. Such responses include release of reactive oxygen species (ROS) and cell wall appositions (CWAs). A successful pathogen will have its own ROS detoxification mechanisms to manage this inhospitable environment. We are studying one such candidate mechanism in the rice blast fungus, *Magnaporthe oryzae*, governed by a gene we call *MoHYR1*, which encodes a glutathione peroxidase domain. Its yeast homolog is a thioredoxin-dependent peroxidase that forms disulfide bonds with a partner protein, YAP1, to regulate ROS detoxification. The wild type *MoHYR1* gene partially complemented the yeast mutant, but it was not rescued by the gene mutated in the cysteines that form bonds with YAP1. A *MoHYR1* deletion mutant showed growth inhibition in hydrogen peroxide (H2O2) and a decreased ability to break down plant-generated ROS during compatible interactions, including ROS associated with CWAs, resulting in significantly smaller lesions on barley and rice. Our results indicate that the *MoHYR1* gene functions similarly to its yeast homolog; it is important for fungal tolerance of H2O2, which is directly related to virulence.

597. Withdrawn

598. Secreted protein from human fungal pathogens use an RxLR-meditated strategy for entering the host cell. Vincenzo Antignani[1], Shiv Kale[1], Brett Tyler[1]. [1]Virginia Bioinformatics Institute, Virginia Polytechnic Institute Blacksburg, USA.

Humans are colonized by different fungal species and most of the time colonization is harmless under normal circumstances. However, during immunosuppression, opportunistic pathogenic fungi have the capability to proliferate and eventually cause a disease. Effectors molecules are a key weapon used by pathogens to evade or circumvent host defense mechanisms in order to succeed in the pathological event. Using a bioinformatic approach, we have identified candidate RxLR-like motifs in three fungal predicted effectors from the human pathogens *Aspergillus flavus*, *Cryptococcus neoformans* and *Coccidioides immitis*. In these effectors we identified double RxLR-like motifs, and both the RxLR sequences are involved in the phospholipid, phosphatidylinositol-3-phosphate (PI-3-P) binding. Using a targeted mutagenesis approach we demonstrated that just one of the two RxLR is critical for the traffic across the membrane of mammalian cells. These observations lead us to speculate that one of the RxLRs is strictly required for the human cell uptake while the other one is mainly involved in membrane anchor process. Theoretically we hypothesize the presence of a primary and of a secondary RxLR with the latter mainly involved in the initial adsorption of the effector proteins around PI-3-P rich membrane domains to enhance the effective concentration of the protein at the specific membrane sites facilitating its penetration.

599. Towards engineering novel resistance against oomycete and fungal pathogens by interfering with phosphoinositide mediated entry of effectors. Julio C. Vega-Arreguin, Shiv D. Kale, Ryan D. Anderson, John M. McDowell and Brett M. Tyler Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA., Department of Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA. Email:jvega@vbi.vt.edu

Recent evidence suggests that oomycete and fungal effectors enter the plant cell via phosphoinositide binding. RXLR and RXLR-like motifs in these effectors were shown to be required for entry in soybean leaves and root cells. E. coli expressed proteins that specifically bind to the phosphoinositide PI3P were able to block effector entry. Based on these previous results from our lab, here we aim to design and test different strategies to protect plants against oomycete and fungal pathogens by targeting the phosphoinositides of the surface of the host cell. Using Agrobacterium-mediated transient expression we established a system for secretion of PI3P-binding domains and other proteins to the apoplast. The addition of the PR1a secretory leader directed most of the proteins to the apoplast of N. benthamiana. Furthermore, the addition of a fluorescent tag allowed us to monitor the expression and localization of the secreted proteins in planta. Using this system we are testing the stability of diverse secreted PI3P-binding domains as well as their ability to block pathogen growth in N. benthamiana. PI3P-binding domains fused to mCherry showed high stability in the apoplast, where they can be detected more than two weeks after agro-infiltration, allowing pathogen assays for longer periods of time. Stable transformants of A. thaliana are also being analyzed for resistance to different pathogens. Additionally, we are expressing non-secreted PI3P- and PI4P-binding domains in planta to analyze phosphoinositide distribution in the cell and its re-organization upon pathogen attack.

600. Functional analysis of Target of Rapamycin kinase during infection-associated autophagy of rice blast fungus Magnaporthe oryzae. Min He, Michael J Kershaw and Nicholas J. Talbot School of Biosciences, University of Exeter, Geoffrey Pope Building, Stocker Road EX4 4QD, United Kingdom

The rice blast fungus Magnaporthe oryzae elaborates a specialized infection structure called the appressorium to breach the plant cuticle before host tissue colonisation. Recent evidence has shown that infection-associated macroautophagy is required for conidial programmed cell death and subsequent appressorium maturation, but how macroautophagy is initiated and regulated during appressorium development remains unclear. Nutrient starvation induces autophagy in both yeast and mammals through inhibition of the Target of Rapamycin (TOR) kinase. TOR is an evolutionarily-conserved protein kinase in eukaryotes which plays central roles in coordinating cell growth and autophagy, in response to environment nutritional status. Inducible-RNA silencing of the TOR kinase gene suggested that it is essential for viability of M. oryzae, and temporal and spatial expression analysis using a TOR(p):sGFP promoter fusion showed that TOR is constitutively expressed in the conidium and appressorium during plant infection, and also in vegetative hyphae grown in nutrient rich medium. Apppressorium-specific over-expression of TOR is not sufficient to inhibit autophagy occurring in the appressorium, indicating the involvement of other potential regulatory components in M. oryzae. Rapamycin, a well known inhibitor of TOR kinase, exerts its effects on growth of M. oryzae by forming a FKBP12-rapamycin-TOR kinase complex, but has an inhibitory effect on conidial autophagic cell death, possibly through inducing cell cycle progression. Taken together, our preliminary data demonstrate the involvement of TOR kinase in infection-related development of M. oryzae, but also suggest alternative genetic control of infection-associated autophagy.

601. Transcriptome analysis of small secreted protein genes in the wheat stem rust fungus, *Puccinia graminis*. Szabo, Les J.[1], Bharti, Arvind, K.[2], Farmer, Andrew D.[2], Crow, John A.[2], Ramaraj, Thiru[2], Yin, Chuntao[3], Hulbert, Scot[3], Sakthikumar, Sharadha[4] and Cuomo, Christina A.[4]. [1]USDA ARS Cereal Disease Laboratory, St. Paul, U.S.A. [2]National Center for Genomic Research, Santa Fe, U.S.A. [3]Department of Plant Pathology, Pullman, U.S.A. [4]Broad Institute, Cambridge, U.S.A.

Puccinia graminis f. sp. *tritici* (Pgt), the causal agent of wheat stem rust, is an obligate biotrophic plant pathogen. In order to better understand the interaction between this fungal pathogen and its hosts, we are characterizing the repertoire of small-secreted protein (SSP) genes. The annotated genome of Pgt contains approximately 1,000 SSP, of which the majority is species-specific when compared with other sequenced fungal genomes. Gene expression has been analyzed using Nimblegen microarrays and Illumina RNA sequencing. Microarray analysis included RNA samples from urediniospores, germinated urediniospores, wheat and barley infected with Pgt reference strain (75-36-700-3, race SCCLC) as well as wheat infected with 6 additional strains of Pgt representing predominant North American races. Twelve different RNA samples from infected wheat and barley were sequenced which included: Pgt reference strain; four representative North American Pgt strains (races HKHJ, MCCF, QCCJ, and TPMK), three members of the North African Ug99 family (races TTKSK, TTKST and TTTSK); and a strain of P. graminis f. sp. secalis. Host sequences were filtered out using wheat and barely EST gene indexes. Transcript assemblies from the filtered sequence reads were constructed and compared the to Pgt assembly. Characterization of transcript levels and genetic variation of SSPs will be presented.

Education

602. Applying Writing Pedagogy to College Biology Laboratory Assignments. Marilee A. Ramesh. Department of Biology, Roanoke College, Salem, VA 24153. ramesh@roanoke.edu

The ability to effectively communicate through writing is an essential skill for our profession and a skill that we are challenged to develop in our students. Writing is not only about communication, but serves as a process to develop and clarify our ideas. Science writing poses its own unique set of hurdles. Writing is expected to be clear and concise, while following a particular format unfamiliar to most undergraduates. While most writing pedagogy has strong foundations in the humanities, some strategies can be adapted for writing assignments in the sciences. Students, using data collected in a laboratory course, are routinely expected to analyze and present their findings in the form of laboratory reports. However, such writing assignments can be constructed to incorporate pedagogical elements such as informal writing, drafting, peer review, conferencing and reflection. Whenever possible, links can be made between pedagogical approaches and real work tasks a professional scientist is expected to perform. Such assignments provide students with the opportunities to improvement in their work and thus, enable them to gain confidence in their writing skills.

603. Incorporating student research in a new seminar program for non-scientists. Virginia K. Hench, Martha S. Arnold and Patricia J. Pukkila University of North Carolina at Chapel Hill, Chapel Hill, NC 27599 USA ghench@med.unc.edu

Our primary goal is to enable non-science majors to gain confidence in their abilities to adopt a scientific approach to complex problems. We are developing a new series of seminar courses in which graduate students and postdoctoral fellows are involved as Graduate Research Consultants (GRCs) or “coaches” for course research projects. This approach builds on our seven years of experience with the GRC program and 413 research-exposure courses enrolling over 12,800 students. Ongoing evaluation has revealed benefits for undergraduates (improved reasoning, increasing the complexity and creativity in what students attempt, reducing student anxiety), for graduate students (gaining confidence and experience, seeing the “inner workings” of courses) and for faculty (using GRCs from other disciplines to provide “insider perspective”, being energized by the student projects, redesigning old courses). Over 80% of faculty report that the program is “significant” or “transformative” for specific student learning goals, and 93% of faculty report that they will use the GRC model again. This program is supported in part by a grant to UNC-Chapel Hill from the Howard Hughes Medical Institute through the Precollege and Undergraduate Science Education Program.

604. Withdrawn

605. COMGEN: Fungal genetic analysis as a pedagogical tool. Bangera, M. Gita [1], Andrea Gargas [2]. [1] Life Sciences, Bellevue College, Bellevue WA, USA. [2] Symbiology LLC, Middleton WI, USA.

COMGEN (Community College Genomics Research Initiative), developed with funding from NSF CCLI #0717470, is an innovative program that teaches the skills of self-directed learning, critical thinking, and analysis. COMGEN provides a mini-graduate school experience for community college students, including the frustrations and delights of scientific discovery. Starting only with basic biology (prerequisite is a single cell biology course), students learn the essential skills of graduate-level researchers. Students perform original research, troubleshoot experiments, organize lab meetings and journal clubs, and learn to network within the greater scientific community. Students choose between two tracks of research. In Track 1, students partner with the USDA, ARS Root Disease and Biological Control Research Unit including Drs. Linda Thomashow and Dave Weller, to sequence the genomic library of a patented biocontrol agent protective against wheat Take-All disease, caused by *Gaeumannomyces graminis* var. *tritici*. In Track 2 students partner with mycologists including Dr. Martha Christiansen, Professor Emerita, University of Wyoming to produce DNA-based characterization of fungal collections with existing morphological, ecological or biochemical data. This sequence information is essential to complete, and in some cases to correct, the identification and phylogenetic analysis of these collections.

606. MycoCosm, a fungal genomics resource for comparative fungal genomics. Aerts, Andrea, Alan Kuo, Bobby Otillar, Robert Riley, Igor Shabalov, Henrik Nordberg, Inna Dubchak, Igor V. Grigoriev. US DOE Joint Genome Institute, Walnut Creek, California, USA

Over 50 fungal genomes have been sequenced, annotated and analyzed by the DOE Joint Genome Institute. These genomes, along with externally-sequenced genomes and analytical tools for comparative analysis can be found in MycoCosm (jgi.doe.gov/fungi), the JGI's fungal genomic resource. MycoCosm integrates this large volume of data with tools for genome-centric analysis and community annotation and has been recently updated with improved comparative tools and tutorials to facilitate their use. A demonstration of these new features will be presented.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

Other

607. *DXS* is responsible for the biosynthesis of destruxins in *Metarhizium robertsii* ARSEF 2575. B. Giuliano Garisto Donzelli[1],·S. B. Krasnoff[1], A. C. L. Churchill[2], D. M. Gibson[1]. [1] R. W. Holley Center for Agriculture and Health, USDA-ARS, Tower Road, Ithaca, New York 14853 [2]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, New York, 14853.

Destruxins are a family of cyclic depsipeptides that include > 35 members produced by Ascomycete fungi belonging to several different taxa. These metabolites display a plethora of biological activities including toxicity against insects, depolarization of the Ca2+ gradient across the plasma membrane and negative effects on both cellular and humoral immune responses in insects. Strains belonging to the genus *Metarhizium* are copious producers of destruxins which have been traditionally considered as likely contributors to virulence. We identified a nonribosomal peptide synthetase gene *DXS* from the *M. robertsii* ARSEF 2575 genome that is predicted to produce a 7,884 aa protein harboring six complete modules. It is expressed both *in vitro* and during pathogenesis of the host *Spodoptera exigua* (beet armyworm, BAW). Independently-generated mutants produced by *Agrobacterium*- mediated gene knockout did not produce detectable DTXs but were otherwise comparable in phenotype to the wild type strain. Preliminary pathogenicity tests indicated that the *DXS*-null mutants were as virulent as the wild type strain.

608. Targeting cellular antioxidation with benzo analogs as a means to increase efficacy of antifungal drugs. Jong Kim, Kathleen Chan, Bruce Campbell. Western Regional Research Center, PMR, USDA-ARS, 800 Buchanan St., Albany, CA 94710.

Resistance to antifungal drugs requires continuous development of new agents. Phenolics are potent redox cyclers and disrupt fungal oxidative stress response systems. Many phenolics do not have significant medical side effects and, thus, have potential use in antimycotic chemotherapy. Benzo analogs were tested against strains of *Aspergillus* and *Penicillium expansum*, agents of invasive aspergillosis and/or producers of mycotoxins. Structure-activity analysis revealed antifungal activity of the analogs increased with a hydroxyl group at position 2 of the aromatic ring. Assays with mutants of *Saccharomyces cerevisiae* (*sod1*delta, *sod2*delta, *glr1*delta) and *A. fumigatus* [*sakA*delta, *mpkC*delta, two mitogen-activated protein kinase (MAPK) mutants], showed antifungal activity was by disruption of cellular antioxidation systems and that *sakA* and *mpkC* genes play overlapping roles to this stress. Microarray and qPCR analyses of *S. cerevisiae* treated with the benzo analog 2,3-dihydroxybenzaldehyde (2,3-D) revealed changes in gene expression depicting a response to chelation of iron/metals and disruption of cell wall integrity. Co-application of 2,3-D, as a chemosensitizing agent to debilitate the fungus, with certain antifungal drugs resulted in a synergistic interaction. This greatly elevated drug efficacy. Use of such chemosensitizing agents to lower drug dose in chemotherapy of human mycoses is discussed.

609. Elucidating mechanisms of telomere instability in *Magnaporthe oryzae*: retrotransposons running riot. Olga Novikova, John Starnes, David Thornbury, Mark Farman Department of Plant Pathology, University of Kentucky, Lexington, KY 40546, USA farman@email.uky.edu

Magnaporthe oryzae strains from perennial ryegrass (prg) have unusually unstable telomeres that continually rearrange during vegetative growth. Sequencing of chromosome ends revealed that the telomeres of the prg pathogens contain insertions of telomere-targeted retrotransposons (MoTeRs) and genetic studies provide evidence that these elements are responsible for the observed instability. Characterization of newly-formed telomeric restriction fragments revealed that the rearrangements occur via several mechanisms, including: *de novo* MoTeR insertions, expansion/contraction of MoTeR arrays, truncation of MoTeR sequences, and duplication of non-telomeric sequences at chromosome ends. Interestingly, the most frequent causes of rearrangement were insertions of the MAGGY retrotransposon into MoTeR sequences. We propose that the MoTeRs cause telomere instability by compromising the telosome, leading to chromosome breaks and further rearrangement via ectopic recombination. Further, we postulate that the MAGGY elements are attracted by telomeric heterochromatin that is propagated across MoTeR arrays.

610. Strain Improvement for the Mass Production of the Fungal Secondary Metabolite Phleichrome from a Phytopathogenic Fungus *Cladosporium phlei*. Moon-Sik Yang[1], Min-Hee Yi[1], Jung-Mi Kim[1], Yong-Seob Jeong[2], and Dae-Hyuk Kim[1]. [1]Institute of Molecular Biology and Genetics, Center for Fungal pathogenesis, [2] Food Science and Technology Major, Chonbuk National University, Jeonju, Korea 561-756

The genetic manipulation of fungi has been suggested as an efficient preparation method for the sustainable production of phleichrome, a derivative of 4,9-dihydroxy-3,10-perylenequinone and a member of a group of fungal perylenequinones. In the present study, UV-mutagenesis was performed to obtain mutant strains that demonstrate altered phleichrome production, the secondary metabolite of *Cladosporium phlei*. UV doses to achieve a 90% kill rate (10% survival rate) were applied to the spores, and pigment production on potato dextrose agar (PDA) plates was visually screened. Fifty mutants were selected based on the increased area and intensity of the purple pigment surrounding the colonies. Thin-layer chromatography (TLC) was applied to determine the phleichrome content in the ethylacetate extract of mycelial agar blocks, and the mutant strain M0035 was selected. The M0035 strain displayed a growth rate and a mycelia mass comparable to the parental strain, but significantly reduced asexual sporulation. The production of phleichrome from the M0035 strain was elevated more than seven fold, and the phleichrome yields from the mycelia and culture filtrates were estimated to be 311 mg/L and 45 mg/L, respectively. Plate cultures with 25 agar plates made from 1 L of liquid media resulted in a total of 592 mg phleichrome consisting of 146 mg and 446 mg from the mycelia and agar media, respectively. These results suggest that culture conditions are important for the production of phleichrome and that higher levels of phleichrome can be obtained from a simple solid culture.

611. Gravity-responsive genes in mushroom formation of the basidiomycete *Pleurotus ostreatus*. Yasumasa Miyazaki[1], Masahide Sunagawa[1], Akira Higashibata[2], Noriaki Ishioka[2], Katsuhiko Babasaki[1], and Takashi Yamazaki[2]. [1] Department of Applied Microbiology, Forestry and Forest Products Research Institute, Tsukuba, Japan. [2] Institute of Space and Astronautical Science, Japan Aerospace Exploration Agency, Tsukuba, Japan.

In mushrooms, the characteristic morphological development, namely, fruiting body formation, is required for sexual reproduction. In response to a change in the direction of gravity, morphogenetic changes of fruiting bodies of mushrooms are usually referred to as gravitropism. This response is similar to that observed in plants. Although gravitropism in fungi has been studied for over 100 years, there is no convincing evidence regarding the graviperception mechanism in mushrooms. To understand gravitropism in mushrooms, we isolated differentially expressed genes in *Pleurotus ostreatus* (oyster mushroom) fruiting bodies developed under simulated microgravity by using three-dimensional (3D) clinostat, an apparatus nullifying the effect of gravity. The subtractive hybridization, cDNA representational difference analysis (cDNA-RDA), resulted in an isolation of 36 individual genes (17 upregulated and 19 downregulated genes) under the condition with clinorotation. These gravity-responsive genes are likely involved in potential cellular mechanisms during fruiting body formation. The phenotype of *P. ostreatus* fruit bodies developed under simulated microgravity vividly depicted the gravitropism in mushrooms.

612. Hyphal Heterogeneity in *Aspergillus niger*. Charissa de Bekker, Arman Vinck, Han Wösten Microbiology and Kluyver Centre for Genomics of Industrial Fermentations, Utrecht University, The Netherlands

Mycelia of filamentous fungi explore new substrates by means of growing hyphae. These hyphae secrete enzymes that degrade organic material into small molecules that can be taken up to serve as nutrients. Previously, it has been shown that only part of the exploring hyphae of *Aspergillus niger* highly express the glucoamylase gene *glaA*. This was a surprising finding considering the fact that all hyphae were exposed to the same environmental conditions. Using reporter studies, we have demonstrated that the expression of other secretion enzyme encoding genes in *A. niger* is also heterogenic. Co-expression studies showed that hyphae that highly express one of these genes also highly express other genes encoding secreted proteins. Over and above this, high expression of genes encoding secreted proteins correlated with high expression of a gene involved in central metabolism and with high ribosomal RNA content. This suggests that there are populations of hyphae at the periphery that differ in their transcriptional and translational activities. These studies were extended with whole genome transcription profiling of individual hyphae. In order to perform (sub)-cellular transcriptomics on single exploring hyphae, protocols have been set up to collect individual hyphae using LPC, isolate RNA and amplify cDNA. Microarray analysis led to the conclusion that exploring neighboring hyphae are highly heterogenic in gene expression. Genes with heterogenic expression can be found in all functional gene classes.

613. The LaeA-like methyltransferase (LlmF) plays a role in secondary metabolism and development in *Aspergillus nidulans*. Jonathan Palmer[1], Jeffery Theisen[1], Rocio Duran[2], Scott Grayburn[2], Ana Calvo[2], and Nancy Keller[1]. [1] University of Wisconsin, Madison, WI 53706 [2] Northern Illinois University, DeKalb, IL 60115

The trimeric velvet complex, consisting of VelB/VeA/LaeA, has been shown to mediate development and secondary metabolism in response to light. While exact functions have not been assigned to members of the complex, proper response to light is in part determined by the cellular location of VeA. Briefly, under illumination VeA is localized mainly in the cytoplasm while in the absence of light VeA is exclusively nuclear. Here we describe a putative methyltransferase LlmF (LaeA-Like Methyltransferase F) and report that it is a negative regulator of secondary metabolism and sexual development. Strains lacking *llmF* produce increased levels of secondary metabolites in a velvet complex dependent fashion while over-expression of *llmF* represses sexual development. We show that LlmF is required for proper cellular localization of VeA, possibly through direct protein-protein interactions as LlmF-VeA interact in a yeast- two-hybrid assay. Further validation of the LlmF-VeA interaction is underway as well as biochemical characterization of LlmF function.

614. Cellobiose as an Inducer of Cellulases in *Neurospora crassa* Elizabeth Znameroski[1], Jordan Tsai[2], Jamie Cate[1,2,3], N. Louise Glass[4] [1]Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720, USA. [2]Department of Chemistry, University of California at Berkeley, Berkeley, CA 94720, USA [3]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA. [4]Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, CA 94720, USA

In the wild, *Neurospora crassa* is often found growing upon recently burned plant matter. Its ability to depolymerize and metabolize plant cell walls through the secretion of cellulases was reported more than 30 years ago, but until recently, very little characterization was performed. This study has begun to examine the molecular mechanism of cellulase induction in *N. crassa*. While the natural product of cellulases, cellobiose, does not act as an inducer molecule in wild type *N. crassa*, it does induce cellulase expression in knockout strains lacking the predicted major beta-glucosidases. This specific induction can be measured as both an increase in cellulase gene transcription as well as secreted enzyme activity. For the most part, previous studies have found that the best inducer of plant cell wall degrading enzymes is the insoluble plant cell wall material, however, in this study, beta-glucosidase knockout strains induced with cellobiose result in a transcriptional response (CBHI and EG2) greater than the wild type response to avicel.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

615. Assessment of conserved function of conidiation regulators in two model ascomycetes, *Aspergillus nidulans* and *Neurospora crassa*. Da-Woon Chung, Srijana Upadhyay, Charles Greenwald, Shengli Ding, Heather H. Wilkinson, Daniel J. Ebbole, and Brian D. Shaw. Department of Plant Pathology and Microbiology, Program for Biology of Filamentous Fungi (PBoFF), Texas A&M University.

Conidia are common asexual spores for fungal reproduction. A number of studies have elucidated the regulation of conidiation in two model ascomycetes, *Aspergillus nidulans* and *Neurospora crassa*. These organisms are evolutionarily distant and their conidiation is morphologically distinct. To understand the evolutionary origins of asexual sporulation, it is critical to examine the conserved function of conidiation regulatory genes between these two species. We concentrated our interests on seven major genes – *fluG*, *flbC*, *flbD*, *abaA*, *wetA*, *medA*, and *stuA* that are known as conidiation regulators in *A. nidulans*. Disruption of the genes resulted in conidiation defects in *A. nidulans*, but the degree of the defects in conidia production and conidiophore formation varies. By analysis of sequences of the *A. nidulans* conidiation genes, we identified seven orthologs in *N. crassa*. Disruption of *flbC*, *medA*, and *stuA* orthologs leads to macro-conidiation defects, whereas *fluG*, *flbD*, *abaA*, and *wetA* orthologs are not associated with macro-conidiation in *N. crassa*. Expression of *N. crassa fluG*, *flbD*, *wetA*, *medA*, and *stuA* orthologs complemented the conidiation defect for the corresponding *A. nidulans* mutant. This implies that the biochemical functions of these genes are conserved between the two species. In contrast, expression of *N. crassa flbC* and *abaA* did not complement conidiation defects in the corresponding *A. nidulans* mutants. Our data supports the independent evolution in the ascomycetes of conidiation regulatory networks, potentially from a common toolkit.

616. Production and characterization of β-glucosidases from termites and their applicability in bioethanol production. Cristiane A. UCHIMA1, Gaku TOKUDA2, Hirofumi WATANABE3, Katsuhiko KITAMOTO1, Manabu ARIOKA1. 1The University of Tokyo, Japan; 2University of the Ryukyus, Japan; 3National Institute of Agrobiological Sciences, Japan. E- mail: cris.uchima@gmail.com

Worries regarding to the current crisis of climate change and depletion of fossil fuels make the utilization of bioethanol as an attractive option for combating both global warming and less dependence on fossil fuels. Lignocellulose is the most abundant substrate for conversion into fuel. Although cellulosic biomass is difficult to be degraded, it is well-known that termites are efficient decomposers of this material. Since β-glucosidase is essential for cellulose utilization, the aim of this study is to produce, characterize, and compare endogenous β-glucosidases from termites. The enzyme G1NkBG is derived from the salivary glands of the termite *Neotermes koshunensis*, whereas G1mgNtBG1 is from the midgut of the termite *Nasutitermes takasagoensis*. The former was successfully expressed in the filamentous fungus *Aspergillus oryzae*, and the second, in the yeast *Pichia pastoris*. They were purified to homogeneity from the culture supernatants. The effect of temperature and pH on β-glucosidases activity and stability, as well as the substrate specificities, were studied. Kinetic analyses were also performed. Interestingly, G1NkBG activity was stimulated by glucose, which is an unusual characteristic as a β-glucosidase. G1mgNtBG1 displayed relatively high optimum temperature and thermostability. Both were active on cello-oligosaccharides. Having β-glucosidases with one of these characteristics is very interesting for biotechnological applications, and they can be useful in bioethanol production. Uchima et al., Applied Microbiology and Biotechnology, in press.

617. Marker recycling in *Aspergillus oryzae* by application of the Cre/loxP system using direct introduction of Cre recombinase into the cell. Osamu Mizutani[1], Kazuo Masaki[1], Katsuya Gomi[2], Haruyuki Iefuji[1]. [1]National Research Institute of Brewing, Hiroshima, Japan, [2]Tohoku University, Sendai, Japan. E-mail : mizutani@nrib.go.jp

Recently, it was reported that defectiveness in non-homologous end-joining DNA repair factor such as Ku and Lig4 improved homologous targeting in filamentous fungi including *A. oryzae*. Therefore, these host strains are especially useful for not only single but also multiple gene deletions. The selectable marker rescue is one of the important molecular techniques which enable the multiple gene deletions. Cre/loxP recombination system comprises a Cre recombinase that specifically recognizes loxP sequence and catalyses a deletion event between two loxP sites in the same direction. This system has been used for marker gene rescues in various organism including Aspergilli. However, many and time-consuming steps are required to carry out this system, for example, the construction of a Cre-expression plasmid, the introduction of the plasmid and the expression of Cre in the transformant. In this study, we investigated a method to introduce Cre recombinase directly into the cells of *A. oryzae*. Nucleic acids such as the fragment or plasmid were found to act as an enzyme career of Cre recombinase. Mixture of commercial Cre and nucleic acid (e.g., pUG6 plasmid) was introduced to the protoplast of *A. oryzae* by the protoplast-PEG method, resulting in transformants of which a selective marker gene flanked by loxP sites was deleted. Thus, we developed a simple marker rescue method by application of the Cre/loxP system using direct introduction of Cre recombinase with enzyme carrier into the cell.

618. Molecular analysis of the role of the HaHOG1 MAP kinase gene in the response and adaptation of the basidiomycete *Heterobasidion annosum* to stress. Tommaso Raffaello[1,2], Fred O. Asiegbu[1] [1]Department of Forest Sciences, [2]VGSB, University of Helsinki, Finland

The basidiomycete *Heterobasidion annosum (Fr.) Bref.* is a filamentous white rot fungus, considered to be the most economical important pathogen of conifer trees. The stress related HaHOG1 MAP kinase, which is considered involved in the fungal osmotic tolerance, was studied under diverse osmotic stress conditions. The bioinformatic analysis reveals that HaHOG1 gene shows a typical MAP kinase domain with a high level of similarity among basidiomycetes. Phylogenetically, the basidiomycete HOG1-like genes group together in a clade quite separated from the ascomycetes. To assay for functional relevance of the gene during osmotic stress, total RNA was extracted and the expression level of the HaHOG1 transcript was quantified by qPCR. In a parallel study, the full-length HaHOG1 gene was cloned and used for a functional complementation assay in the *S. cerevisiae hog1* mutant strain. The results show that the fungus reveals a decreased growth when exposed to an increased salt osmolarity conditions. Increased levels of the HaHOG1 gene transcript is observed in high stress conditions. The HaHOG1 gene is able to complement the function in the *S. cerevisiae hog1* mutant strain. Taken together these results show the putative role of the HaHOG1 gene in the basidiomycete *Heterobasidion annosum* and its importance in the capacity of the fungus to overcome osmolarity stress conditions in the natural environment.

619. RAT1, a 91aa protein confers sensitivity of *Neurospora crassa* to metabolite(s) secreted by a sponge-associated *Aspergillus tubingensis.* Liat Koch[1], Elazar Cohen[2] Shmuel Carmeli [2] and Oded Yarden [1] [1]The Hebrew University of Jerusalem and [2] Tel Aviv University, Israel Sponge-associate fungi are a promising source of natural products, due to the unique ecological niche in which they reside.

Among 85 fungal taxa previously isolated from the marine sponge *Psammocinia* sp., approximately 10% were identified as *Aspergillus* spp. Strains OY207 and OY907 secreted metabolites that inhibited growth of several fungi (*Alternaria alternata*, *Rhizoctonia solani*, *Neurospora crassa*). The strains were identified, on the basis of partial ITS and beta-tubulin sequences as *A. tubingensis* and *A. insuetus*, respectively. To determine the nature of the inhibitory effects imposed by the secreted compound(s), we generated random tagged *N. crassa* mutants resistant to the *Aspergillus* spp. medium extracts. Plasmid rescue analysis of one of the mutants indicated that a defect in a yet-uncharacterized gene (NCU03140.4), designated *rat-1*, confers resistance to the *A. tubingensis* extract. This was confirmed by analysis of the appropriate knock-out strain and *rat-1* complementation. RAT1::GFP expression indicates it is localized to hyphal septa, suggesting that the toxic compound may function in association with septal proteins/structures. Current analysis indicates that the two *Aspergillus* spp. secret novel terpenoids and polyketides, whose structures and antifungal properties are being assessed.

620. Development of protease deficient Chrysosporium lucknowense (C1) strains. Theo Verwoerd, Vivi Joosten, Hans Visser, and Jan Wery.Dyadic Netherlands, Wageningen, The Netherlands. tverwoerd@dyadic.nl

The filamentous fungus Chrysosporium lucknowense C1 has proven to produce and secrete large quantities of extracellular enzymes in laboratory and industrial scale fermentations. High yields of homologous enzymes or enzyme mixtures are readily obtained. The production of heterologous proteins, however, may sometimes be hampered by the presence of host proteases that partially or fully degrade the heterologous protein. In order to significantly improve the production of “problematic” heterologous proteins, C1 strains are developed that have minimized protease levels without having their vital functions disturbed. To this end different C1 hosts were subjected to random mutagenesis and subsequent screening approaches. Strains with greatly reduced overall protease activity levels were obtained. Additionally, the presence of specific proteases in C1 culture samples was shown by MS analyses. Some of these showed homology to notoriously harmful proteases known from other fungal systems. A protease gene disruption approach was used to knock-out the corresponding genes. This work was greatly facilitated by the use of strains defective in the non- homologous-end-joining of DNA. Thus, a number of versatile low protease C1 hosts were developed for high level expression of heterologous proteins. Some examples will be presented on the poster.

621. Secretome analysis of concentric zones of *Aspergillus niger* colonies. Pauline Krijgsheld 1, A.F. Maarten Altelaar 2, Harm Post 2, Jeffrey H.Ringrose 2, Albert J.R.Heck 2, Han A.B.Wösten 1 1 Microbiology, Utrecht University and Kluyver Centre for Genomics of Industrial Fermentation. 2 Biomolecular Mass spectrometry, Utrecht University and Netherlands Proteomic Centre

Fungi secrete enzymes to convert organic matter substrates into small molecules. Hyphae at the periphery of the colony are exposed to unexplored organic material, whereas the center of the colony experiences a utilized substrate. This suggests that the enzymes that are secreted by different zones in the colony are different. Spatial protein secretion was monitored by growing *Aspergillus niger* colonies between two porous polycarbonate membranes that had been placed on top of agar medium containing xylose as a carbon source. This mode of growth inhibits sporulation and forces the colony to grow two-dimensionally. After 6 days of growth, the colony was transferred to a ring plate. This plate contains 5 concentric wells that are filled with liquid medium. After growing the colony for 24h on the ring plate using xylose as carbon source, medium of the concentric wells was collected. Secreted proteins in each of the wells were labeled and identified by mass spectrometry. The secretome of the colony consisted of 80 proteins. Eleven proteins were less abundant in the colony center compared to the intermediate and peripheral zone, while another five proteins were most abundantly secreted at the periphery of the colony. Taken together, it can be concluded that heterogeneity of the colony is reflected in the spatial secretion pattern. Acknowledgement: This project was financed by the Kluyver Centre for Genomics of Industrial Fermentation and the Netherlands Proteomics Centre, which are part of the NGI

622. Meiotic silencing in *Gibberella zeae.* Hokyoung Son [1] , Kyunghun Min[1], Jungkwan Lee[1], Namboori B. Raju[2], Yin-Won Lee[1*] [1]Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea [2]Department of Biology, Stanford University, 371 Serra Mall, Stanford, CA 94305-5020

Gibberella zeae is a homothallic ascomycete fungus that causes head blight/ear rot in grain crops. The objective of this study is to determine if meiotic silencing occurs in *G. zeae*. The *roa* gene of *G. zeae* affect ascospore morphogenesis and the deletion mutants are partially dominant in asci in outcrosses with wild-type strain. This unusual phenotype is similar to the ascus dominance triggered by meiotic silencing by unpaired DNA in *Neurospora crassa*. We further identified an additional gene, *rsp-1*, which triggered abnormal ascospores when deleted. The *rsp-1* deletion mutant also exhibited partial ascus dominance when outcrossed with strains that carried the wild-type allele. We ectopically inserted an additional copy of *roa* or *rsp-1* into the wild-type strain. Ascus dominance was frequently observed when these duplication strains were outcrossed with strains that carried the wild-type allele. However, the no ascus dominance was observed if the duplication strains were outcrossed with a *sad-1* deletion mutant, which lacks the putative RNA-dependent RNA polymerase essential for meiotic silencing in *N. crassa*. Cytological studies with GFP and RFP-fusion proteins demonstrate that meiotic silencing does exist in the homothallic *G. zeae*, but it is functionally different from that of heterothallic *N. crassa*.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

623. A Novel Gene, *ROA*, Is Required for Normal Morphogenesis and Discharge of Ascospores in *Gibberella zeae*. Kyunghun Min[1], Jungkwan Lee[1], Jin-Cheol Kim[2], Sang Gyu Kim[1], Young Ho Kim[1], Steven Vogel[3], Frances Trail[4], and Yin- Won Lee[1*]. [1] Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea. [2] Chemical Biotechnology Center, Korea Research Institute of Chemical Technology, Daejon 305-600, Republic of Korea. [3] Department of Biology, Duke University, Durham, North Carolina 27708-0338. [4] Department of Plant Biology and Department of Plant Pathology, Michigan State University, East Lansing, Michigan 48824-1312.

Gibberella zeae is a causal agent of Fusarium head blight which is a significant disease among cereal crops including wheat, barley, and rice, due to contamination of grain with mycotoxins. Ascospores of *G. zeae* are forcibly discharged from perithecia forming on crop residues and dispersed. In this study, we characterized a novel gene, *ROA*, which is required for normal sexual development. Deletion of *ROA* (delta-*roa*) resulted in abnormal size and shape of asci and ascospores, however it did not affect vegetative growth. Delta-*roa* strain showed round-shaped ascospores and insufficient cell division after spore delimitation. The asci of the delta-*roa* strain discharged fewer ascospores from the perithecia, but achieved a greater dispersal distance compared to those of the wild-type strain. Turgor pressure within the asci was estimated through the instrumental analysis of osmolytes in the epiplasmic fluid. Deletion of the *ROA* gene appeared to increase turgor pressure in the mutant asci. The higher turgor pressure of the delta-*roa* mutant asci and the mutant spore shape contribute to the longer distance dispersal. When delta-*roa* was outcrossed with delta-*mat1-2*, a strain that contains a green fluorescence protein (GFP) marker in place of the *MAT1-2* gene, unusual phenotypic segregation occurred. The ratio of GFP to non-GFP segregation was 1:1, however, all eight spores had the same shape. Taken together, the results of this study suggest that *ROA* plays multiple roles in maintaining the proper morphology and discharge of ascospores in *G. zeae*.

624. Towards understanding cytochrome P450 & reductase function in the filamentous fungus *Cochliobolus lunatus*. Nada Kraševec[1], Ljerka Lah[1], Metka Novak[1], Branka Korošec[1], Matjaž Vogelsang[1], Mojca Benèina[1], Barbara Podobnik[2], Sabina Berne[3], Jure Stojan[3], and Radovan Komel[1,3]. [1]National Institute of Chemistry, Ljubljana, SLO. [2]Lek Pharmaceuticals d.d., Ljubljana, SLO. [3]Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, SLO. nada.krasevec@ki.si .

Complex biochemistries of filamentous fungi include numerous cytochromes P450. Besides a large number of P450s involved in the filamentous growth and maintenance, there are, as partners, multiple microsomal redox partners - cytochrome P450 reductases (CPRs), associated with these processes. In the pathogenic ascomycete *Cochliobolus lunatus*, two CPR paralogues sharing 34% amino acid identity were identified and recombinantly expressed. Both were reconstituted in the system harboring a benzoate para-hydroxylase (CYP53A15) from the same species. CYP53A15 plays a crucial role in detoxification of benzoic acid and other phenols through the β-ketoadipate pathway of aromatic compounds degradation. The aim of our work was to conduct a comparative study on the functioning of the two reconstituted cytochrome P450–CPR systems and gain insight into the physiological role of the two CPRs. In vivo deletion mutant and complementation studies together with temporal expression profiles of studied genes detected differences in growth on different inhibitor-supplemented media. Live-cell imaging by confocal laser scanning microscopy colocalized the studied proteins in living cells. In vitro biochemical characterization experiments addressed issues regarding redox partner specificity between a particular CPR and CYP53A15, and the effects of a substrate and product specificity. Results hint that one of CPRs plays a role in primary metabolism, whereas the other more in xenobiotic detoxification.

625. Characterization of regulators of G protein signaling in *Gibberella zeae*. Ae Ran Park, Ahram Cho, Kyunghun Min, Jungkwan Lee, and Yin-Won Lee Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea. arpark@snu.ac.kr

Regulator of G protein signaling, RGS, negatively regulates heterotrimeric G proteins that play critical roles to extracellular signals. RGS has approximately 130 conserved amino acids that interacts with activated Galpha subunit to accelerate GTPase activity. In this study, we identified and characterized seven *RGS* genes of *G. zeae*, designated as *GzRGS1* to *7*. *GzRGS1* deletion mutant, delta*gzrgs1*, reduced conidial production and conidial germination of the mutant was faster than that of the wild-type strain in potato dextrose broth (PDB). The conidial production of delta*gzrgs2* was not different from that of the wild-type strain but conidial germination was delayed in PDB. Both delta*gzrgs1* and delta*gzrgs2* strains enhanced zearalenone and deoxynivalenol production. In sexual reproduction, delta*gzrgs1* lost both self- and female-fertility, and the number of discharged ascospores of delta*gzrgs3* and delta*gzrgs4* strains reduced in carrot agar. The length and the number of septa of conidia were decreased in delta*gzrgs5*. Virulence of delta*gzrgs1* through delta*gzrgs3* were significantly reduced. Deletions of *RGS6* and *RGS7* did not result in any phenotype changes. Our study showed that RGS1 to 5 of *G. zeae* regulate vegetative growth, asexual development, sexual development, toxin production and virulence.

626. State of fungal genome resources at NCBI. Barbara Robbertse and Tatiana Tatusova., The National Center for Biotechnology Information, National Institutes of Health Bethesda, Maryland, USA.

NCBI maintain several databases, containing a wide range of information, covering the whole spectrum of life, including fungi. The number of information resources and new databases are growing fast and new functionality is added continuously which could make navigation a challenge. The poster presentation aim to highlight recent additions and the interconnectivity of databases as it pertains to fungal data. Some of the recently added resources include RefSeq Targeted Loci, Peptidome, Biosystems, FLink and Images. At the time of writing the number of Entrez databases reached 40 and the Entrez data retrieval system serves as the main gateway by which to access data with text searches. Sequence based searches are facilitated by the well known BLAST tool, while other tools such as TaxMap, TaxPlot and UniGene DDD help to answer specific questions a user may have. Recently NCBI's eukaryotic gene prediction tool Gnomon has been expanded to process gene predictions of multiple relatively closely related genomes simultaneously. Multi-genome Gnomon has been tested on fungal genomes and this gene prediction service is available to anyone who submits fungal genomes to the public repository at NCBI.

627. *IRE1* plays a critical role in ER-stress response, cell wall integrity, thermotolerance, and virulence in *Cryptococcus neoformans.* Kwang-Woo Jung[a], Seon Ah Cheon[b], Yin-Lien Chen[c], Joseph Heitman[c], Hyun Ah Kang[b], Yong-Sun Bahn[a] [a]Department of Biotechnology, Center for Fungal Pathogenesis, Yonsei University, Seoul, Korea; [b]Department of Life Science, Research Center for Biomolecules and Biosystems, College of Natural Science, Chung-Ang University, Seoul, Korea; [c]Department of Molecular Genetics and Microbiology, Medicine, and Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina

A central component for the UPR pathway is an ER-stress induced kinase-RNase, Ire1. To elucidate the role of the UPR pathway in the human fungal pathogen *C. neoformans*, we identified and characterized the function of the *C. neoformans* Ire1 ortholog. By BLASTp search using the protein sequence of *S. cerevisiae* Ire1 as a query, we identified a single open reading frame, CNAG_03670.2, in the genome database of the serotype A *C. neoformans* strain H99. The predicted protein sequence of CNAG_03670.2 exhibited overall 25% amino acid sequence identity to that of *S. cerevisiae* Ire1. The CNAG_03670.2 ORF encodes a protein of 1,072 amino acids and contains typical Ire1- domain structures such as a luminal domain, a Ser/Thr protein kinase, and a ribonuclease domain. Thus, the CNAG_03670.2 ORF was named the *IRE1* gene in *C. neoformans*. The *ire1* mutants exhibited extreme growth defects at 37¡ÆC and hypersensitivity to ER stress and cell wall destabilization. Finally, Ire1 was shown to be critical for virulence of *C. neoformans*, suggesting UPR signaling as a novel antifungal therapeutic target.

628. Heterogeneity in micro-colonies of *Aspergillus niger* in liquid shaken cultures. G. Jerre van Veluw, Charissa de Bekker, Arman Vinck, L. Ad Wiebenga and Han A.B. Wösten Microbiology and Kluyver Centre for Genomics of Industrial Fermentations, Institute of Biomembranes, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands, H.A.B.Wösten@uu.nl

Colonies of the filamentous fungus *Aspergillus niger* secrete large amounts of proteins. On solid media only part of the colony is involved in secretion. We assessed whether heterogeneity can also be found in micro-colonies of *A. niger*. Strains expressing *GFP* from the glucoamylase (*glaA*) or the ferulic acid esterase (*faeA*) promoter were grown in liquid medium. We used the Complex Object Parametric Analyzer and Sorter (COPAS) to analyze the diameter and fluorescence of the micro-colonies. Two populations of micro-colonies were distinguished that differed in their diameter. The population of small micro-colonies of strains expressing *GFP* from the *glaA* or *faeA* promoter comprised 39 % and 25 % of the culture, respectively. Two populations of micro-colonies could also be distinguished when expression of *GFP* in these strains was analyzed. The population lowly expressing *GFP* consisted of 68 % and 44 % of the whole population, respectively. Here, the COPAS was used for the first time to analyze *A. niger* micro-colonies. Our results show that there is heterogeneity in size and gene expression between micro-colonies within a liquid shaken culture. This implies that protein production in an industrial fermentation can be increased by reducing the heterogeneity between the micro-colonies.

629. Gene expression profiling of an industrial bioethanol *Saccharomyces cerevisiae* strain during fermentative process. Osmar Carvalho-Netto[1], Luciana Mofatto[1], Marcelo Carazzolle[1], Paulo Teixeira[1], Piotr Mieczkowski[2], Juan Lucas Argueso[3], and Goncalo Pereira[1]. [1]Departamento de Genetica e Evolucao, Universidade Estadual de Campinas, Campinas, Brazil. [2]Department of Genetics, University of North Carolina, Chapel Hill, USA. [3]Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, USA.

The bioethanol production system used in Brazil is based on the alcoholic fermentation of sucrose derived from sugarcane feedstock by highly adapted strains of the yeast *Saccharomyces cerevisiae* . We have recently described the complex genome structure of one of the most productive and widely adopted strains: PE-2. In this study we are taking advantage of the available PE-2 genome sequence to investigate the molecular physiology of sugarcane bioethanol fermentation through genome-wide transcription profiling using sequencing-based methods (RNA-Seq), under industrial scale fermentations. The results to be presented here offer new insight into the biology of the PE-2 strain, including the expression behavior of novel genes absent in well-characterized laboratory strains, and the identification of stress response mechanisms active in PE-2 that could explain its superior fitness and competitiveness during bioethanol production. We intend to use these information to devise successful genetic manipulation strategies for the development of further enhanced bioethanol strains.

630. The Aspergillus and Candida Genome Databases (AspGD and CGD), curated gene and protein information resources for the fungal research community. Martha B. Arnaud 1, Jonathan Binkley 1, Gustavo Cerqueira 2, Marcus C. Chibucos 2, Maria C. Costanzo 1, Jonathan Crabtree 2, Diane O. Inglis 1, Joshua Orvis 2, Prachi Shah 1, Marek S. Skrzypek 1, Gail Binkley 1, Stuart R. Miyasato 1, Jennifer Russo Wortman 2, and Gavin Sherlock 1. 1 Department of Genetics, Stanford University, Stanford, CA; 2 Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD arnaudm@stanford.edu

The Aspergillus Genome Database (www.aspgd.org) and the Candida Genome Database (www.candidagenome.org) are web-based genomics resources for researchers studying two important groups of fungi, the Aspergillus and Candida species. We provide high-quality manual curation of the experimental scientific literature, as well as tools for exploring these data, and community resources. The curated information for each gene appears on its Locus Summary page (LSP) with links to pages that provide phenotype and GO details, sequence and annotation history and a comprehensive list of reference citations. As we add multiple species, the LSPs for orthologs are interlinked to facilitate browsing of related genes. At AspGD, we initially curated the gene-specific literature for the model organism Aspergillus nidulans, and now also for the pathogen A. fumigatus. At CGD, we have curated Candida albicans and are now curating C. glabrata. In the future, we will expand our efforts to other species, including A. niger and A. oryzae for AspGD, C. dubliniensis and C. parapsilosis for CGD. AspGD and CGD are supported by NIH RO1 AI077599 from the NIAID and R01 DE015873 from the NIDCR, respectively.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

631. Proportion of mating type specific nuclei and their associated gene expression in *Neurospora tetrasperma.* Nicklas Samils and Hanna Johannesson. Department of Evolutionary Biology, Uppsala University, Sweden. E-mail: nicklas.samils@ebc.uu.se

Neurospora tetrasperma is a pseudohomothallic ascomycete that normally packs haploid nuclei of both mating- types in each of the ascospores following meiosis. Each ascospore gives rise to a heterokaryotic and self-fertile individual. We studied the ratio between mat-A and mat-a nuclei in *N. tetrasperma* in different tissues and life stages in three heterokaryotic wild type individuals. The design was based on pyrosequencing of strain- and mating type-specific SNPs. Both total DNA and RNA were extracted from the samples thus allowing ratio correlation between nuclear DNA and gene expression of the investigated genes. Mat-A nuclei were strongly dominating young mycelia in quantity (dominated 67-99% of the examined young material, with an average of about 87%). Perithecia formation and initiation of ascospore production increased the ratio of mat-a nuclei. However, the general trend in tissues with fewer perithecia showed a higher number of mat-A nuclei. These findings suggest that the mat-A nuclei grow or move faster within the individual and therefore dominate the young mycelia, while the mat-a nuclei moves slower, but are essential for meiosis. The mating type specific gene expression was found linear to the ratio of the template DNA, indicating that the ratio of parental template DNA plays a role in quantitative mating type specific gene expression.

632. Identification of a Novel Genetic Locus in Aspergillus fumigatus. Jennifer C. Jackson and Xiaorong Lin Biology Department, Texas A&M University, 3258 TAMU, College Station, TX 77843-3258 jcjackson@tamu.edu

In order to discover novel genetic loci in Aspergillus fumigatus, a library of 8,000 random insertional mutants of A. fumigatus has been generated via Agrobacterium-mediated transformation and screened for various defects in conidial color. One mutant of particular interest produces conidia of a light green, turquoise color and appears to have altered cells surface protein expression. The specific site of T-DNA insertion in this selected mutant has eluded identification using inverse PCR. Use of the A. fumigatus AMA1 genomic library is being used to complement the mutant phenotype and identify the interrupted gene. Using the wax moth model, Galleria mellonella, this mutant has elicited an extreme immunoresponse in the host compared to the wild type A. fumigatus, Af293. This result is similar, but more extreme, than that observed previously with A. fumigatus albino, pksP mutant causing rapid death and altered immune response in the wax moth host. Scanning Electron Microscopy reveals the surface of the condia of the mutant are markedly smooth compared to that of the wild type under the same conditions. The mutant phenotype with the wax moth and SEM data suggests that the presence of gene mutation causes the increased expression of an immune dominant antigen on the conidial surface of the mutant. Alternatively, the gene mutation may cause an absence or non-production of a conidial surface protein, exposing an underlying protein in its absence. Obviously more can be elucidated once the genotype has been identified.

633. Functional characterization of genes regulated by G protein alpha subunit 1 in *Gibberella zeae.* Jungkwan Lee [1], Chanju Park [1], Jin-Cheol Kim [2], Jung-Eun Kim [1], and Yin-Won Lee [1] [1] Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea. [2] Korea Research Institute of Chemical Technology, Daejon 305-600, Korea.

We previously reported that G protein alpha subunit 1 (GPA1) is essential for sexual reproduction in *Gibberella zeae*. In this study, we performed microarray analyses on a GPA1 deletion mutant to identify genes involved in the sexual reproduction of this fungus. In the mutant, 645 genes and 550 genes were down- and up-regulated, respectively, during sexual reproduction compared to the wild-type strain. Quantitative real time-PCR was used to determine transcriptional profiles of one hundred of the down-regulated genes at various sexual and vegetative stages. Transcript levels of 78 of these genes were dramatically increased in the wild-type strain during sexual reproduction compared to levels observed during vegetative growth, and were down-regulated in the mutant compared to the wild-type strain. We deleted 57 of these genes and found that four of the deletion mutants could no longer self fertilize and five of the mutants produced fewer perithecia compared to the wild-type strain. Two mutants produced wild-type numbers of perithecia, but maturation of perithecia and ascospores was delayed. In all we identified 11 genes that are involved in sexual reproduction of *G. zeae* and present evidence that some of these genes function at distinct stages during sexual reproduction in the fungus.

634. Optimizing the RNA-Seq transcriptome discovery pipeline for *Aspergillus fumigatus.* Suman Pakala, Vinita Joardar, Nikhat Zafar, Suchitra Pakala, Sean Murphy, Natalie Fedorova and William Nierman J. Craig Venter Institute, Rockville, MD, USA. spakala@jcvi.org

RNA-Seq has become invaluable to applications such as transcript expression quantification and genome annotation, including discovery of non-coding RNAs and identification of new gene models and isoforms. The continued evolution of deep sequencing technologies has resulted in active development of new data analysis tools. It has become necessary for research groups to identify the appropriate set of tools that best suit their needs. As part of the *Aspergillus fumigatus* re-annotation project, we sequenced several cDNA libraries using Illumina GA II and evaluated publicly available transcript discovery approaches. Specifically, this comparison includes (i) the de novo transcript assembly approach, (ii) the alignment followed by assembly of transcripts approach and also (iii) a hybrid approach for transcript identification. Performance of various short read aligners, splice junction mappers, and transcript assemblers that implement these approaches have been evaluated. Based on this study, we present a general framework for evaluating RNA-Seq data analysis tools and discuss our results of such an evaluation and optimization of the analysis pipeline for the *A. fumigatus* genome.

635. *Gzmyb1*: A Novel Gene Involved in Fruiting Body Development of *Gibberella zeae.* Yang Lin, Hokyoung Son, Kyunghun Min, and Yin-Won Lee Department of Agricultural Biotechnology and Center for Fungal pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea, linyangleona@gmail.com

Gibberella zeae (anamorph: *Fusarium graminearum*) is the cereal pathogen of wheat, barley, corn and rice. Ascospores, forcibly discharged from mature fruiting bodies (perithecia), serve as the primary inocula for disease epidemics. In this study, we identified the disrupted gene of a Restriction Enzyme Mediated Integration (REMI) mutant (Z39P105), which was unable to produce mature perithecia, by tail-PCR. This gene, named *Gzmyb1*, contains a myb DNA binding domain and the green fluorescence protein fused with this protein was localized in nuclei, suggesting it is a putative transcription factor. The *Gzmyb1*-deleted mutant showed same phenotypes to the wild-type strain in vegetative growth, asexual reproduction, conidial germination, virulence, mycotoxins production, and the response to stress, but it was unable to produce mature perithecia. Further when the deletion mutant was outcrossed with a heterothallic strain that carries the deletion of *mat1-2*, it had male-fertility but not female-sterility, indicating that Gzmyb1 is involved in the development of perithecia. To identify genes regulated by Gzmyb1, we performed microarray analysis using RNA of the deletion mutant and the wild-type strain isolated after sexual induction. Transcripts that showed the greatest changes were found to belong to genes included in posttranslation modification, energy production and carbohydrate, amino acid, lipid and secondary metabolites biosynthesis transport and metabolism. Thus, Gzmyb1, a putative transcription factor, was shown to be specifically required for fruiting body development in *G.zeae*.

636. Exploration of biosynthetic genes for fusaric acid in *Fusarium oxysporum*. Theresa Lee, Seung-Ho Lee, Jean Young Shin, Soohyung Lee, Mija Kim, Jong-Chul Yun, Jae-Gee Ryu Microbial Safety Division, NAAS-RDA, Suwon, 441-707, Korea

Fusaric acid (FA) is a mycotoxin produced by *Fusarium* species. Its toxicity is low but often associated with other mycotoxins, thus enhancing total toxicity. To date, biosynthetic genes or enzymes for FA have not been found or isolated. Therefore, restriction enzyme mediated insertional (REMI) mutagenesis has been applied to FA producing *F. oxysporum* strains in order to knock out FA biosynthetic gene(s). REMI of two stains tested has yielded more than 2,000 mutants with efficiencies of 3.1 and 13.8%, respectively. To set-up mutant screening system using phytotoxicity of FA, various grains and vegetable seeds were tested for germination in cultures where FA-producing strain was grown. Among eleven seeds, only Chinese cabbage seeds were not germinated in the culture showing sensitivity to FA, thus selected for further screening of the REMI mutants. Each mutant isolate was grown in Czapek-Dox broth at 25C with vigorous shacking for 3 weeks then culture filtrate was filtered into a petri dish containing 20 cabbage seeds. Each container was incubated at 25C under continuous fluorescence light and monitored for seed germination at 24 and 48 hours. Among about 550 mutants tested, at least six appeared that the cabbage seeds were germinated at 48 hours but the level of germination was lower than medium only. This result suggests that FA production by the mutants were reduced rather than eliminated, requiring further analysis.

637. Analysis of the secretomes of *Cryptococcus gattii* strains with different virulence profiles. Leona T. Campbell[1], Elizabeth Harry[2], Ben Herbert[3] and Dee A. Carter[1]. [1]School of Molecular Bioscience, University of Sydney, Sydney, NSW, Australia [2] iThree Institute, University of Technology, Sydney, NSW, Australia [3]Department of Chemistry & Biomolecular Sciences, Macquarie University, Sydney, NSW, Australia

Cryptococcus gattii is capable of causing disease in a wide range of animal hosts. Closely related strains of *C. gattii* exhibit significant differences in virulence in mammalian hosts. As fungi produce a range of secreted degrative enzymes, and as these may invoke a host response, the fungal secretome is likely to be important in modulating host- pathogen interaction. In this study, we compare the secretomes of two *C. gattii* strains, one categorized as hypervirulent (R265) and the other exhibiting low-level virulence (R272). A total of 27 proteins were identified with only four proteins being shared between strains. The secretome of R265 primarily included uncharacterized proteins containing catalytic cores with roles in carbohydrate degradation as well as the antioxidant superoxide dismutase and a GTPase. R272 secreted a more diverse set of proteins including enolase and transaldolase, enzymes canonically involved in glycolysis and the pentose phosphate pathway respectively, but both also described as fungal allergens that bind IgE. This work indicates that different classes of proteins are secreted by closely related strains of *C. gattii* exhibiting different levels of virulence.

638. Mutagenesis of *sdhB* and *sdhD* genes in *Botrytis cinerea* for functional analysis of resistance to SDHIs. Anaïs Lalève[1,2], Anne-Sophie Walker[1], Pierre Leroux[1], Valérie Toquin[2], Hélène Lachaise[2]& Sabine Fillinger[1] [1]UR BIOGER-CPP, INRA avenue Lucien Brétignières F78850 Thiverval-Grignon France, [2]Bayer SAS, BayerCropScience, La Dargoire Research Center 14-20 rue Pierre Baizet F69009 Lyon France anais.laleve@versailles.inra.fr

Botrytis cinerea is a phytopathogenic ascomycete responsible for grey mould on many crops. Respiratory inhibitors play an increasing role in the control of this disease. Succinate dehydrogenase inhibitors (SDHIs, including carboxamides) inhibit the fungal respiration by blocking the ubiquinone-binding site of the mitochondrial complex II. Old SDHIs (i.e. carboxin), essentially active against Basidiomycetes were replaced in the 2000s by a new generation of SDHIs with a broader spectrum including Ascomycetes. Boscalid is the only representative of this new generation in France up to now. A few years after its market introduction, field mutants of *B. cinerea*, resistant to boscalid were isolated in France and Germany. At least six different phenotypes, named CarR1 to CarR6, have been pinpointed by characterizing their resistance pattern towards 20 SDHIs. CarR1 to R4 phenotypes exhibit low to medium level of resistance, whereas CarR5 and R6 show high level of resistance to different SDHIs, including boscalid. CarR1 and CarR2 strains are currently the most frequent strains detected in German and French vineyards. The resistance mechanism was investigated for the different phenotypes by searching for putative alterations in the SDH proteins, which could be responsible for the observed resistance. Our findings show that excepted for CarR2, point mutations occurring in the *sdhB* gene lead to a specific amino acid change in SDHB for each phenotype. For CarR2 strains, we distinguished at least 3 sub-groups: strains with i/ a point mutation in the *sdhB* gene, ii/ a point mutation in the *sdhD* gene and iii/ no mutation in any of the four *sdh* genes. In order to confirm the role of these mutations in the various SDHI resistance phenotypes, we have generated isogenic mutants through a gene replacement strategy. In addition the isogenic strains will allow us to estimate in how far CarR resistant strains are adapted to field conditions and may represent a risk for growers.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

639. Interaction between Streptomycetes and Aspergillus nidulans. Hans-Wilhelm Nützmann[1 & 5], Volker Schroeckh[1], Kirstin Scherlach[2], Wolfgang Schmidt-Heck[3], Karin Martin[4], Christian Hertweck[2 & 5], and Axel A. Brakhage[1 & 5]; 1 Department of Molecular and Applied Microbiology, 2 Department of Biomolecular Chemistry, 3 Systems Biology/Bioinformatics Group, 4 Bio Pilot Plant Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute (HKI) and & 5 Friedrich Schiller University Jena. HKI Jena, Beutenbergstrasse 11a, 07745 Jena, Germany hans-wilhelm.nuetzmann@hki-jena.de

Microorganisms as bacteria and fungi produce many important low-molecular weight molecules that show different biological activities. Genome mining of fungal genomes indicated that their potential to produce these compounds designated secondary metabolites is greatly underestimated. However, most of the fungal secondary metabolism gene clusters are silent under laboratory conditions. Therefore, a major challenge in this emerging area is to understand the physiological conditions under which these compounds are produced. Results in this area will lead to the discovery of new bioactive compounds and to new insights in fundamental aspects of communication between microorganisms. To address these questions the important model fungus Aspergillus nidulans was coincubated with 58 different Streptomycetes. With one particular species, a specific interaction was shown. For the first time, using microarray analyses at the molecular level it was demonstrated that this interaction leads to the specific activation of two distinct silent secondary metabolism gene clusters. Electron microscopy confirmed the intimate interaction of the fungus and the bacterium. Full genome arrays of A. nidulans were applied to elucidate the whole genome response to the streptomycete. Data on the molecular regulation of the involved secondary metabolism gene clusters will be presented.

640. Premature polyadenylation of heterologous genes in *Ustilago maydis.* Johannes Freitag and Michael Bölker, Philipps-Universität Marburg, Department of Genetics

Premature polyadenylation of foreign genes has been observed in various fungal species including *U. maydis*. We use *U. maydis* as a model to understand the principles of polyadenylation. Therefore we developed an *in vivo* approach which allows the identification and quantification of polyadenylation events simply by measuring GFP- fluorescence. The characterisation of downstream regions of *U. maydis* as well as bioinformatic analysis revealed that there seems to be no consensus sequence for polyadenylation signals. Very redundant AT enriched sequences triggered polyadenylation. Additionally, we found that sequences in genes from *Saccharomyces cerevisiae* could act as polyadenylation signals in *U. maydis* without exception. We suggest that in *U. maydis* no classical polyadenylation signals exist but the nucleotide composition of the open reading frames prevents polyadenylation. This could be a means to guard the genome of *U. maydis* against invasive DNA such as viruses and transposons.

641. Evaluation of *Penicilium* Echinulatum secretome through Shotgun Proteomics. Daniela A. Ribeiro, Hans Muller Paul, Beatriz M. P. Pereira, Jose G. C. Pradella, Roberto Ruller, Adriana F. Paes Leme, Fabio Squina. Centro Nacional de Pesquisa em Energia e Materiais, Brazil.

Penicilium echinulatum has been considered a promising cellulolytic fungus for several biotechnological applications. Based on secretome analysis and biochemical characterization, we evaluated its biomass degrading protein repertoire. The fungus was grown using five different types of biomass: crude bagasse, cellulose, bagasse derived from steam-explosion treatment, derived from sulfuric acid treatment or hydrothermal treatment. The secretome was analyzed by shotgun LC-MS/MS and enzymatic activity of culture supernatant was tested using several commercial polysaccharides. Search against Fungi database identified over than 100 proteins, such as different glycoside hydrolases(GH), accessory proteins (including swollenin), and a number of predicted or hypothetical proteins. It is interesting to highlight that the hydrolytic activity pattern observed corroborated with the proteomic analysis. For instance, the highest hydrolytic activity using p-nitrophenyl (PNP) glucopyranoside substrate was found in secretome derived from crude sugarcane bagasse and micropulverized cellulose, in which GH3 beta-glucosidases were identified by mass spectrometry. Indeed, specific hydrolytic activities observed using PNP cellobiose, CMC, pectin, xylan and other substrates were also correlated to the amount and types of GH counterparts identified by MS/MS. Our approach revealed to be a powerful tool for development of enzymatic cocktails for biofuels production.

642. What is the impact of the two channel proteins Mid1 and Cch1 on transient increases of cytosolic Ca^{2+} in *Botrytis cinerea*? Karin Harren and Bettina Tudzynski IBBP, WWU Münster, Schlossgarten 3, 48149 Münster, Germany, karin.harren@uni-muenster.de

Eukaryotic cells respond and adapt to environmental stress by a network of signal transduction pathways which are essential for survival. A variety of cellular processes are mediated through transient increases of cytosolic Ca^{2+}. Those arise from internal stores like the vacuole or the ER but can also derive from influx via the plasma membrane. In *S. cerevisiae* two channel proteins are described to be the major players in maintaining the intracellular Ca^{2+} level: Mid1 a putative stretch-activated Ca^{2+} channel, and the voltage-gated high- affinity Ca^{2+} channel Cch1. The cellular impact of external Ca^{2+} will be investigated by functional characterization of deletion mutants of the corresponding homologs in the necrotroph *B. cinerea*. Both deletion mutants (*bcmid1* and *bccch1*) and the double knockout mutant exhibit similar phenotypes, as they are generally not impaired in vegetative growth and morphology on standard media. In addition to that, these deletion mutants had no effect on virulence. However, under high salt conditions and with the Ca^{2+}-chelator agent EGTA vegetative growth is affected. Furthermore, pH stress causes restricted growth in axenic culture. Recently generated mutants expressing Aequorin, an intracellular Ca^{2+} reporter, produce a whole pool of available information on the effect of these two channels on the intracellular Ca^{2+} concentration. For further investigations, localisation studies with GFP-fusion proteins for both channel proteins are in progress.

643. Transcriptional and genomic profiling of industrial *Trichoderma reesei* strains Susanna Mäkinen[1], Marja Paloheimo[1], Tiina Pakula[2], Mikko Arvas[2], Merja Oja[2], Markku Saloheimo[2], Merja Penttilä[2], Jarno Kallio[1], Jari Vehmaanperä[1] and Terhi Puranen[1]. [1] Roal Oy, P.O.B 57, 05200 Rajamäki, Finland [2] VTT, P.O.B 1000, 02044 VTT, Finland. E-mail: susanna.makinen@roal.fi

Trichoderma reesei has a long history in industrial enzyme production because of its high protein secretion capability and easy processability. Despite its many positive features, further strain engineering is still being pursued to remove any remaining protein production and secretion bottlenecks. Systems biology methods were applied to study protein production in selected proprietary *T. reesei* strains. Transcriptional analysis using microarrays was performed to identify differentially expressed genes in various cultivation conditions and high-density Comparative Genomic Hybridization (CGH) was used to characterize genomic alterations in the strains. Combined data from CGH and transcriptional analyses was utilized to identify target genes for further improvement of protein production/secretion. Deletions and/or overexpressions of 16 genes involved in protein transport, post-translational modification, basic energy metabolism or regulatory functions were studied in detail.

644. Co-cultivations of fungi improve the production of specific enzymes. Isabelle Benoit (1), H.L. Hu (2,3), J. van den Brink (4), B. S. Gruben(1), J.D. Gu (2), H.A.B. Wösten (1) and R.P. de Vries(1,4) (1)Microbiology & Kluyver Centre of Genomics for Industrial Fermentation, Utrecht University, Utrecht, The Netherlands; Email: i.benoit@uu.nl. (2)Division of Microbiology, University of Hong Kong, Hong Kong SAR, People's Republic of China. (3)CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands.

During their natural life cycle most fungi encounter other microorganisms and live in mixed communities with complex interactions, such as symbiosis or competition. Industrial fermentations, on purpose or by accident, can also result in mixed cultures. Fungal co-cultivations have been previously described for the production of specific enzymes, however, little is known about the interactions between two species that are grown together. *A. niger* and *A. oryzae* are two of the most important industrial fungi worldwide and both have a long history of strain improvement to optimize enzyme and metabolite production. Co-cultivation of these two Aspergilli with each other and with the ascomycete phytopathogen *Magnaporthe grisea*, and the basidiomycete white rot fungus *Phanerochaete chrysosporium*, has recently been described by our group (Hu et al, 2011). Total secreted protein, enzymatic activities related to plant biomass degradation and growth phenotype were analyzed from cultures on wheat bran. Data from this paper and follow-up studies will be presented. Reference Hu et al. (2011) Int. Biodeterioration and Biodegradation, Article in Press (doi:10.1016/j.ibiod.2010.11.008)

645. Transcriptional analysis of *Trichoderma reesei* cultivated in the presence of different lignocellulose substrates. Mari Häkkinen , Markku Saloheimo, Merja Penttilä and Tiina Pakula VTT Technical Research Centre, P.O.Box 1000 (Tietotie 2, Espoo), Fin-02044 VTT, Finland; mari.hakkinen@vtt.fi, tel. +358 20 722 111, fax. +358 20 722 7071

Trichoderma reesei is a soft rot Ascomycete fungus able to secrete enzymes extremely efficiently. Production strains have been traditionally improved by classical mutagenesis as well as by specific genetic modifications. The availability of the complete genome sequence of *T. reesei* has made it possible to utilise genome wide analysis methods to study physiology and protein production by the fungus at different conditions and to utilise the information obtained to develop new strains with better enzyme production qualities, such as capability for enhanced protein production or production of modified enzyme mixtures for degradation of specific types of lignocellulose materials. In this study the hydrolytic system of *Trichoderma reesei* Rut-C30 in the presence of different substrates was studied by cultivating the fungus in the presence of e.g. sophorose, cellulose, pretreated wheat straw, pretreated spruce, xylan and bagasse. The cultures were subjected to transcriptional profiling using oligonucleotide microarrays. Differentially expressed genes were indentified from the data and expression profiles of glycoside hydrolase genes and other genes encoding lignocellulose degrading enzymes were compared in the presence of the different substrates to identify co-regulated groups of genes.

646. Redox-sensitive GFP2: A biosensor of the redox status in *Botrytis cinerea.* Jens Heller[1], Andreas Meyer[2] and Paul Tudzynski[1] [1]IBBP, WWU Münster, Schlossgarten 3, D-48149 Münster, Germany [2]Heidelberg Institute for Plant Science, Ruprecht-Karls-Universität Heidelberg, Im Neuenheimer Feld 360, D-69120 Heidelberg, Germany jens.heller@uni-muenster.de

There is evidence that the necrotrophic plant pathogen *B. cinerea* has to cope with oxidative stress during the infection process caused by an early plant defense reaction, the oxidative burst. However, to answer the question if *Botrytis* suffers oxidative stress during the infection process, we are in need of tools to measure its redox status in vivo. The ratiometric redox-sensitive GFP2 (roGFP2) reversibly responds to redox changes induced by incubation with H_2O_2 or DTT in vitro and specifically senses the glutathione redox potential (E_{GSH}) after expression in different organisms. Here we show that roGFP2 is also functional in *Botrytis* hyphae and can be used to analyze kinetics of GSH recovery after H_2O_2 treatment in different strains. It was shown recently that in *B. cinerea* the AP1-transcription factor Bap1 is the main transcriptional regulator of H_2O_2- scavenging proteins. However, it is not essential for the virulence of *B. cinerea*. Our data suggest that although Bap1 controls the genes for GSH recovery on transcriptional level, the *bap1* deletion mutant and the wildtype do not differ in the kinetics of GSH recovery. This indicates that other so far unidentified factors might be more important for the regulation of the glutathione system during oxidative stress in *B. cinerea*.

647. Structural and functional analyses of the mating type (*MAT*) locus of the dimorphic ascomycetous animal pathogen *Blastomyces dermatitidis*. Thomas D. Sullivan[1], Wenjun Li[2], Joseph Heitman[2] & Bruce S. Klein[1] [1]Department of Pediatrics, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53706 [2]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710
The teleomorph of *B. dermatitidis* can be generated by *in vitro* pairing of opposite mating type strains in a heterothallic, bipolar system, leading to the formation of cleistothecia containing eight-spored asci. In the euascomycetes, sexual reproduction is governed by the interaction of alpha-box containing *MAT1-1* strains with HMG domain- containing *MAT1-2* strains. We found that the region surrounding *MAT 1-2* in three *B. dermatitidis* strains contains up to 40 kb of inserted DNA, not found in the single *MAT1-1* idiomorph analyzed. We also validated that the *MAT* loci alpha-box and HMG-domain genes are linked to mating type determination in *B. dermatitidis*. To accomplish this we determined the mating types from F1 progeny of a fertile mating, by crossing each to *MAT* tester strains, and compared the results to the results of PCR assays specific for either the alpha-box or the HMG-domain gene. In all cases the PCR results were concordant with the mating type determined genetically. Marker analysis in the F1 progeny demonstrated that recombination, and therefore, meiosis had occurred. These findings open the door to classic genetic analysis and studies on the role of mating in virulence.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

648. The first large-scale EST project for *C. passeckerianus*, the pleuromutilin producing fungus. Kate de Mattos-Shipley, Gary Barker, Gary Foster and Andy Bailey. Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK. E-mail: Kate.deMattos-Shipley@bristol.ac.uk .

Pleuromutilin derivatives are economically and medicinally important compounds. Tiamulin and Valnemulin have been used in veterinary medicine for many years, Retapamulin is currently used to treat impetigo and other Staphylococcal skin infections in humans, and various new derivatives are currently in clinical trials with the potential of being orally available. Despite this importance, little has previously been known concerning the biosynthesis of pleuromutilin and the biology of the pleuromutilin producing fungus, *Clitopilus passeckerianus*. Recent studies of *C. passeckerianus* and pleuromutilin have elucidated the taxonomy of pleuromutilin producing fungi and have reported the generation of a molecular toolkit for the genetic manipulation of this fungus. This poster will present the first large-scale sequencing project for *C. passeckerianus* including the generation and analysis of Expressed Sequence Tags from fruiting bodies, a pleuromutilin-producing fermentation culture and a non-producing fermentation culture. Implications for the regulation of Pleuromutilin biosynthesis are discussed.

649. Secrelection: A Novel Fungal Expression System For Selection Of Secreted Enzymes. Robbert Damveld [1], Brenda Vonk [1], Cees Sagt [1], Hildegard Menke [1], Thibaut Wenzel [1]. [1]DSM Biotechnology Center

Modern biotechnology has generated an impressive set of molecular tools: for instance the ability to generate large sets of error prone mutant libraries or cDNA libraries. When these libraries are expressed in a host (e.g. *Aspergillus niger*) not all strains produce a secreted protein. This is mainly dependent on the quality of the library. Here we describe a novel expression system that was developed by using genome expression profiling under different conditions. We were able to identify promoters that fit the required expression profile. These promoters were both up regulated during protein secretion and were not expressed during overexpression of intracellular proteins. By making use of transcriptomics for useful promoter identification, we were able to generate reporter construct(s) that allow us to easily select clones that secrete proteins. This technology can speed up novel protein discovery significantly. Additionally we have shown this approach is not limited to fungi but can also be applied to other production organisms.

650. Fungal enzymes for biomass deconstruction. Suzana Car, Goutami Banerjee, John S. Scott-Craig, Melissa S. Borrusch, Jonathan D. Walton Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, Michigan, USA.

Enzymes for biomass deconstruction are a major cost in the production of ethanol from lignocellulosic biomass. Currently available commercial enzyme cocktails have generally been optimized for acid-pretreated stover from corn and other grasses and are therefore unlikely to be well-adapted for all of the pretreatment/biomass combinations that exist now and that will emerge in the future. In order to understand better which enzymes, and in what proportions, are optimal for biomass deconstruction, we have developed a high-throughput analysis platform called the GLBRC Enzyme Platform (GENPLAT). We have used this platform to optimize synthetic enzyme cocktails on a variety of pretreated feedstocks, and to evaluate more than 18 fungal enzymes in order to understand their roles in biomass deconstruction. To date, all of our enzymes are derived from *Trichoderma reesei*, but many other fungi contain enzymes that might contribute to biomass degradation. We are using GENPLAT and our synthetic mixtures to "bioprospect" for novel accessory enzymes and superior key enzymes. This poster summarizes our effort to better understand the immense potential and roles of fungal enzymes in deconstructing lignocellulosic biomass, with the ultimate goal of contributing to the development of a practical lignocellulosic ethanol industry.

651. Genome-wide identification of replication origins in *Candida albicans*. Meleah Hickman, Hung-Ji Tsai, Amnon Koren, Laura Burrack, and Judith Berman

DNA replication is an essential biological process and the necessary machinery is well conserved among eukaryotes. However, the genomic features that specify origins of replication (ORIs) and replication timing are not well understood. Current replication timing data supports a stochastic origin-firing model where the most efficient origins are in fact the earliest firing. We have found that centromeres are highly efficient, constitutive ORIs in *Candida albicans*. These centromeric origins not only bind the origin replication complex (ORC) with high affinity; they also have asymmetric GC skew patterns. These skew patterns are well conserved at centromeres in other *Candida* species as well as the distantly related *Yarrowia lipolytica* and indicate that the centromeric origins are extremely old. Non-centromeric ORIs are difficult to identify because there are many more ORC binding sites than active origins, and most ORIs do not appear to be constitutive. We are using comparative genomics with the yeasts *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Schizosaccharomyces pombe* to identify features associated with replication origin activity in order to characterize active, non-centromeric ORIs in *C. albicans*. Alignment of ORC binding sites with genome-wide nucleosome position revealed a strong correlation between ORC binding and nucleosome-depletion. Furthermore, a 50 bp motif recently identified in *K. lactis* as an ARS (autonomously replicating sequence) consensus sequence (ACS) appears in a subset of ORC binding sites in *C. albicans*. Currently, our results suggest that active replication origins in *C. albicans* fire in an evolutionarily conserved manner based on sequence features and chromatin-dependent factors.

652. Oxidative stress response and immunogenicity of catalase-peroxidase in Penicillium marneffei. Monsicha Pongpom, Aksarakorn Kummasook, Nongnuch Vanittanakom. Department of Microbiology, Faculty of Medicine, Chiang Mai University, Thailand.

Penicillium marneffei is an important opportunistic fungal pathogen in Southeast Asian countries. Ability to survive inside macrophages of host is believed to be important in establishment of infection. Previously, we have isolated a gene encoding a catalase-peroxidase (CpeA) from P. marneffei by antibody screening of a cDNA library. The CpeA transcript is preferentially expressed during growth in pathogenic yeast phase at 37 oC and in phase conversion from mold to yeast. Moreover, hydrogen peroxide treatment induced drastic upregulation of the transcript. These results suggested that CpeA has a crucial role in heat and oxidative responses. Since the CpeA gene was isolated by antibody screening approach, we performed heterologous expression of catalase-peroxidase in E. coli and used the expressed protein in western blot analysis with human sera to test its immunogenic property. Antibodies to the recombinant protein were found in all 15 sera that obtained from P. marneffei-infected AIDS patients. Pooled sera obtained from normal healthy people, both from Thailand and non-endemic region did not show reactivity. In addition, none of sera from patients with candidiasis, aspergillosis, histoplasmosis, cryptococcosis and tuberculosis reacted to the recombinant protein. These results suggested a potential use of this protein as the diagnostic marker to detect infections due to P. marneffei.

653. Fungal Biodiversity of Arid Ecosystems of Baja California. Adriana Romero-Olivares1, Raúl Baptista-Rosas1 and Meritxell Riquelme2 1Autonomous University of Baja California, Graduate School of Molecular Ecology and Biotechnology and 2Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada CICESE, Ensenada, Baja California, Mexico. Adrilu.romero@gmail.com

Little is known about fungal soil inhabitants, their distribution and their relationship with the rest of the components of the ecosystems they inhabit (Fierer, 2007). Fungi can exist under many environmental conditions; this includes arid ecosystems, which are distinguished by annual precipitations of less than 254 mm of water (Bhatnagar, 2005). Baja California (BC), Mexico, has arid ecosystems in more than 50% of its territory, with an annual precipitation ranging between 100 to 200 mm of water (INEGI: National institute of statistics and geography of Mexico). These ecosystems have a unique physiography and climatology that vary along the state (Delgadillo, 1990). Therefore to conduct a prospective analysis of fungal biodiversity in these arid ecosystems, soil samples were taken from these locations and total genomic DNA was extracted. An ITS gene library was constructed and clones were selected for identifying RFLPs and subsequent sequencing. We calculated diversity indices followed by phylogenetic analyses. Our results showed that the fungal biodiversity of the state follows biogeographic patterns. The majority of the phylotypes found correspond to non-identified fungi. We conclude that in BC, fungal biodiversity is practically unknown, and the few identified species are distributed depending on the climatic, physiographic and biogeographic characteristics of the locations sampled.

654. Diverse Pathways Generate Aberrant RNAs, MicroRNA-like RNAs and Dicer-Independent Small Interfering RNAs in Fungi. Heng-Chi Lee[1], Liande Li[1], Antti Aalto[2], Weifeng Gu[3], Qiuying Yang[1], Craig Mello[3], Dennis Bamford[2], Yi Liu[1]. [1]Department of Physiology, University of Texas Southwestern Medical Center, Texas, USA. [2]Institute of Biotechnology and Department of Biosciences, University of Helsinki, Finland. [3] Program in Molecular Medicine, University of Massachusetts Medical School, USA.

In addition to small interfering RNAs (siRNAs) and microRNAs, several types of endogenously produced small RNAs play important roles in gene regulation. In the filamentous fungus *Neurospora*, the production of qiRNAs requires QDE- 1, QDE-3 and Dicers. Surprisingly, our results suggest that the RNA dependent RNA polymerase (RdRP) QDE-1 is also a DNA-dependent RNA polymerase that produces DNA damage-induced aberrant RNAs (aRNAs), which are the precursor for qiRNA biogenesis. By comprehensively analyzing small RNAs associated with the Argonaute protein QDE-2, we also show that diverse pathways generate miRNA-like small RNAs (milRNAs) and Dicer-independent small interfering RNAs (disiRNAs). milRNAs are processed by at least four different mechanisms that use a combination of Dicers, QDE-2, the exonuclease QIP and a novel RNAse III domain-containing protein MRPL3. disiRNAs originate from loci producing overlapping sense and antisense transcripts, do not require any of the known RNAi pathway components for their production. Taken together, several novel pathways are uncovered, shedding light on the diversity and multi-evolutionary origins of eukaryotic small RNAs.

655. The *Aspergillus niger* Multicopper Oxidase Family. J.A. Tamayo Ramos[1], S. Barends[2], R. Verhaert[2] and L.H. de Graaff[1]. [1]Fungal Systems Biology, Laboratory of Systems and Synthetic Biology, Wageningen University, Dreijenplein 10, 6703 HB, Wageningen, The Netherlands. [2] ProteoNic bv, Niels Bohrweg 11-13, 2333CA Leiden, The Netherlands E-mail: *juan.tamayoramos@wur.nl*

Multicopper oxidases (MCOs) form a family of enzymes that is widely distributed in nature. These enzymes play different biological roles in e.g. lignification and delignification processes, transport of ions or cell division and morphogenesis (1,2). This family of enzymes includes laccases, ascorbate oxidases, metal oxidases and bilirubin oxidases, which are evolutionary and structurally related (3) but have different substrate affinity and specificity. The ability of these enzymes to catalyze reactions by producing just water as the only by-product has increased the interest of the industry for their use as 'green' catalysts (4). Their importance is reflected in the broad spectrum of reported applications, that range from pulp delignification, textile dye bleaching and water or soil detoxification, to the formation of pigments, the development of clinical tests and applications in the field of biosensors, bioreactors, and biofuel cells (1). Despite the wide interest for this class of enzymes, these have not been characterized yet in the filamentous fungus *Aspergillus niger*. This in contrast to the many efforts to over-express MCOs from other sources in black aspergilli like *A. niger* (5, 6, 7). Therefore, in order to find out if *A. niger* could be a good source of new multicopper oxidases with possible biotechnological interest, the MCO putative genes present in the genome of *A. niger* N593 strain were identified and their phylogenetic relationships determined. According to the *in silico* analysis of their coding products, those genes identified as good candidates to produce MCOs were cloned and overexpressed in *A. niger*. The overexpressed MCOs showed, in plate assays, different patterns of activity towards a number of laccase substrates, suggesting that they have different specificity and therefore different biochemical characteristics. Further studies will be done in order to show the potential biotechnological use of the novel *A. niger* MCOs. 1)*Chem Rec* 7: 220-9. 2)*Trends Biotechnol* 28: 63-72. 3)*FEBS J* 273: 2308-26. 4)*Trends Biotechnol* 24: 219-26. 5)*Appl Environ Microbiol* 67: 2610-6. 6)*J Biotechnol* 134: 9-19. 7)*Appl Biochem Biotechnol* 129-132: 195-214

656. Spatial localization of protein secretion machinery components in *Magnaporthe oryzae* biotrophic invasive hyphae Martha C. Giraldo[1], Yasin Dagdas[2], Nicholas J. Talbot[2] and Barbara Valent[1]1Dept. of Plant Pathology, Kansas State University, Manhattan, KS, USA.[2]School of Biosciences, University of Exeter, Geoffrey Pope Building, Exeter, U.K.

To cause disease, the rice blast fungus undergoes a dimorphic switch between the filamentous invasive hyphae (IH) that first grow inside rice cells and bulbous IH that subsequently colonize them. Secreted effector proteins accumulate in the biotrophic interfacial complex (BIC), which forms at the tip of each filamentous IH and remains beside the first differentiated bulbous IH cell. FRAP analysis (fluorescence recovery after photobleaching) demonstrated that effectors continue to accumulate in BICs as the bulbous IH proliferate in the rice cells. We have localized fluorescent fusions of the Spitzenkörper-associated proteins MoMlc1p (myosin regulatory light chain) and MoSnc1p (a v-SNARE) and the polarisome-associated MoSpa2p during IH invasion of rice cells. Localization of both Spitzenkörper and polarisome markers in the filamentous IH initially resembles localization patterns in *M. oryzae* vegetative hyphae *in vitro*. After differentiation to bulbous IH, the Spitzenkörper is not, however, observed at hyphal growth points. Instead, a Spitzenkörper-like structure was observed as an intense fluorescent spot in the first bulbous IH cell, generally near to the BIC. The polarisome marker MoSpa2p shows a spatially distinct localization pattern from MoMlc1p and MoSnc1p in the bulbous IH. These results provide the first evidence that BIC-associated bulbous IH cells are undergoing active exocytosis, consistent with a role for BICs in the delivery of effectors during rice tissue invasion.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

657. Functional screening for cellulolytic activity in Australian wood-inhabiting fungi. Jess Welch, Evette Steele, Geoff Dumsday, Victoria Haritos and Brendon Monahan. Energy Transformed Flagship, CSIRO, Parkville, Victoria, 3168

Plant biomass forms the largest waste stream in the world, incorporating waste from industries including agriculture, paper and pulp, forestry and textiles. The major component of this biomass is the recalcitrant glucose polymer cellulose. The breakdown of cellulose into its component sugars provides both a waste-disposal solution and a sustainable feedstock for the production of alternative energy sources such as ethanol. Enzymatic hydrolysis of cellulose to sugars for fermentation is currently the rate-limiting step in industrial bioethanol production, due to the considerable amounts of enzyme required and the relatively slow rate of conversion. More efficient cellulose-degrading enzymes (cellulases) are essential for making the production of ethanol from sustainable feedstocks an economically-viable option for liquid transport fuels. The CSIRO Australian wood-inhabiting fungal collection was established in 1930 and contains 2150 isolates. The collection spans a wide taxonomic range of fungi and has not previously been explored for the presence of cellulases. Here we describe a functional screen of this collection for growth on a range of cellulosic substrates. A number of fungi with notable cellulase activity were subsequently identified for further characterisation. Ribosomal ITS sequencing was used for species identification and cellulase genes were isolated using reverse genetics and/or degenerate PCR approaches. Functional characterisation of isolated cellulase genes by recombinant expression is currently underway. Overall, a functional screen of the CSIRO Australian wood-inhabiting fungi collection has identified cellulolytic isolates with potential for industrial application.

658. Apoptotic fungal cell death mediates host invasion by pathogenic fungi. Amir Sharon, Neta Shlezinger, Sagi Shimshoni, Adi Doron. Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv 69978 Israel

The gray mold fungus *Botrytis cinerea* is a wide host-range plan pathogen causing significant crop losses worldwide. Most of the damage is due to post harvest rotting and infection of wounded plant tissues. Infection of intact plants is less significant, at least partly due to sensitive of the fungus to plant-derived anti-fungal compounds. During disease spreading, the fungus induces cell death in the host and inhabit only already killed tissues, thus avoiding direct contact with toxic plant metabolites. How the fungus mange to survive during the initial phase, when the spores are in contact with live host cells remains unclear. We hypothesized that apoptotic cell death (PCD) might be induced in the fungus by plant defense metabolites during the initial phase of disease establishment. Here we report on the characterization of PCD in *B. cinerea* and the role of the fungal anti-apoptotic response in disease development.

Bioinformatics searches revealed presence of a wide range of candidate apoptotic genes in fungi, but also absence of homologues of several important regulators of animal apoptosis. We isolated and characterized BcBir1, a *B. cinerea* homologue of IAP proteins, due to the central position of IAPs in mammalian apoptotic networks. Knockout or over expression strains of BcBIR1 revealed that BcBir1 is anti-apoptotic and this activity was assigned to the N' terminal part of the protein. Using a strain expressing GFP-tagged nuclei and direct apoptosis assayed we found that the fungus undergoes massive programmed cell death during early stages of infection, but then fully recovers upon transition to second phase of infection. Further studies using the fungal mutants in combination with mutant Arabidopsis lines showed that fungal virulence was fully correlated ability of the fungus to cope with plant-induced PCD. Similar results were obtained with another necrotrophic pathogen *Cochliobolus heterostrophus*. Our result show that BcBir1 is major regulator of PCD in *B. cinerea* and that proper regulation of the host-induced PCD is essential for pathogenesis in this class of pathogens. Due to the general role of PCD in fungi and considering the common strategies of host invasion by pathogens, we propose that host-induced fungal PCD might be a general phenomenon including in human pathogens. The components of apoptotic networks, although only partially characterized, are conserved between fungi but differ from plant and animals. When considered together, it is expected that apoptotic networks might represent attractive targets for novel antifungal drugs.

659. Protoplast from *Aspergillus sojae* ATCC20235: isolation and regeneration. Amira M. Rizk[1,2] and Marcelo Fernandez-Lahore[1]. [1]Downstream Processing Laboratory, Jacobs University Bremen gGmbH, Campus Ring 1, D-28759, Bremen, Germany. [2]Food Science and Technology Department, Faculty of Agriculture, Tanta University, Egypt

As a result of the convenience applications of protoplasting tool including classic and modern genetic manipulation, it is important to optimize a system to produce a high a mount of viable protoplast. This work carried out to study the main factors affecting the formation, purification and regeneration of protoplasts in *Aspergillus sojae* ATCC20235. Lytic enzymes, digestion medium pH, osmotic stabilizers, mycelial age and amount, were examined in order to find the optimal protoplast yield. Also the factors affecting protoplast regeneration had been evaluated. The highest amount of protoplast 1.5×10^6 cells/mL was obtained by using lytic enzyme cocktail (lytic enzyme from Trichoderma harzianum, Chitinase, Hemicellulase and β-glucuronidase). This cocktail optimized to treat 0.01 mg mycelia /ml with 20 h old and the digestion medium pH was 5.5 contains 0.6 M KCL as osmotic stabilizer. The obtained results could be helpful for different approaches such as protoplast fusion, transformation and mutagenesis in term of producing new strains with superior ability to generate valuable compounds for instance enzymes, polysaccharide and pharmaceutical products.

660. A molecular approach to characterize Glomus AMykor isolate: species determination, DNA content establishment and ploidy level analysis. Kinga Anna Sedzielewska[1,2], Dorothee Klemann[1], Joerg Fuchs[1], Nicolai M. Nuerk[1], Katja Vetter[2], Roland Watzke[2] and Gotthard Kunze[1]. [1]Leibniz Institute of Plant Genetics and Crop Plant Research, Corrensstr.3, D-06466 Gatersleben, Germany. [2]AMykor GmbH, Kühlturmstrasse 25.34.00, D-06766 Bitterfeld-Wolfen, Germany

The arbuscular mycorrhizal fungi (AMF) are able to establish a symbiotic relationship with 70-90% of land plant species; this interaction has a major impact on the entire soil ecosystem. Although AMF play a key role in the global ecosystem, little is known about them. Problems with their characterization result from a hidden lifestyle in the soil and from the fact that only a few morphological characters can be used for their identification. This resulted in many AM fungi isolates being mistakenly placed in orders, genera or determined as a single species based on a similar morphotype.In our study, standard molecular methods were used to analyse a Glomus industrial strain produced in Germany by the AMykor GmbH using DAOM197198 isolate as a reference organism. To determine the species affinities of the Glomus AMykor isolate the rDNA region (the 3' partial nuclear small subunit SSU, the ITS region and the 5' partial nuclear large subunit LSU) was used for phylogenetic analyses. To establish the nuclear DNA content and the ploidy level of the Glomus AMykor isolate flow cytometry (FC) method was used. Although the FC method allows the estimation of DNA content per nucleus, it does not give a direct answer about the genome size. For this knowledge, the ploidy level of the investigated organism has to be known. Development of fluorescence in situ hybridization (FISH) technique has offered a valuable tool for the ploidy level estimation in several plant species. This encouraged us to use FISH to determine the number of rDNA loci in Glomus AMykor isolates and to potentially use the obtained results for estimation of the genome size and the ploidy level of the analyzed organism.

661. Metagenomics within the cytoplasm: estimating genome diversity in arbuscular mycorrhizal fungi. Eva Boon[1], Sebastien Halary[1], Mohamed Hijri[1], Eric Bapteste[2]. [1]Institut de Recherche en Biologie Végétale, Département de Sciences Biologiques, Université de Montréal, 4101 rue Sherbrooke Est, QC, H1X 2B2, Canada. [2]Université Pierre et Marie Curie (UPMC), Centre National de la Recherche Scientifique (CNRS), Unité Mixte de Recherche 7138, 75005 Paris, France

Measuring genetic diversity in arbuscular mycorrhizal fungi (AMF) is a major challenge, not only for ecologists in the field but also for molecular biologists in the lab. AMF are hypothesized to be heterokaryotic coenocytes, with at least thousands of genetically divergent nuclei that share the same cytoplasm. This peculiar genomic organisation leads to extremely high levels of genetic variation, which up till now has not been sampled exhaustively for a single polymorphic marker. In this study, we use a conservative network-based approach to estimate overall genetic diversity in pyrosequencing runs of three different AMF species (*Glomus irregulare*, *G. diaphanum* and *G. etunicatum*). This diversity is compared to simulated pyrosequencing runs on Candida species. We report high levels of genetic variation, which are organized in patterns that confirm the hypothesized heterokaryotic state of AMF. The use pyrosequencing to sample AMF diversity in the field is discussed.

662. *Colletotrichum higginsianum* genome and EST sequencing to search for effector candidates. Charlotte van der Does, Hiroyuki Takahara, Linda Rincon, Jochen Kleemann, Emiel Ver Loren van Themaat, Richard O'Connell. Max Planck Institute for Plant Breeding Research, Köln, Germany

Colletotrichum higginsianum attacks many cultivated forms of Brassica as well as the model plant *Arabidopsis thaliana*. Following initial penetration by melanized appressoria, biotrophic infection hyphae develop inside living host epidermal cells, before thinner necrotrophic hyphae ramify throughout the tissue. The 52.5 Mb genome of *C. higginsianum* comprises 11 chromosomes and was sequenced with a combination of 454 (24X), Illumina paired-end (60X) and Sanger fosmid end-sequencing (0.2X). These data assembled into 10,235 contigs in 367 scaffolds. Two of the 11 chromosomes are very small (<1 Mb). Since pathogen effector genes can be clustered on mini-chromosomes, genes present on these two chromosomes were identified. With the aid of an optical map, three sequence scaffolds (of ~200 kb in total) could be aligned to one of the mini-chromosomes. Most genes located on these scaffolds were of unknown function and few were predicted to encode secreted proteins. Other genes showed homology to methyltransferases, sugar transporters, transposases, reverse transcriptases and NACHT family proteins. For genome annotation, 890,000 ESTs were generated from *C. higginsianum* by 454-pyrosequencing cDNA libraries representing appressoria in vitro and three stages of plant infection. ESTs from plant-penetrating appressoria and biotrophic hyphae were mined for genes encoding candidate secreted effector proteins. To assign the corresponding genes to a genomic location, chromosomes were separated on a CHEF gel, Southern blotted and hybridized with effector candidate probes. As a first step towards elucidating the function of these effector candidates, proteins were tagged with mCherry for localisation in planta.

663. Transcriptomics of the interactions between wheat and *Septoria tritici* (*Mycosphaerella graminicola*). S. Deller*, C. Audeon, S. Lafarge, J.-P. Pichon, J.-B. Morel, C. Corbeau, T. Marcel, S. Paillard, D. Barloy, M.-H. Lebrun. * INRA BIOGER, avenue Lucien Brétignières, Thiverval-Grignon, 78850

Septoria tritici blotch is an important disease of wheat worldwide. The genome sequence of the pathogen is available, yet its mode of pathogenicity, and the means by which host cells are resistant or susceptible are poorly understood. The TWIST project aims to collect large scale transcriptomic data about different types of interactions (resistant/susceptible) between wheat and *Septoria tritici* (*Mycosphaerella graminicola*). Genome-wide Affymetrix expression arrays are being used to detect genes up- or down-regulated from both the pathogen and the host. Infection time courses have been produced from six wheat cultivars carrying different genetic sources of resistance towards *Septoria tritici* blotch, including cultivars with partial resistance, and others with major resistance genes (STB genes). Time courses of infection (0-24 dpi) have been harvested from resistant and susceptible interactions on young leaves and adult flag leaves (14-51 dpi). The RT-qPCR analysis of classic wheat defence gene expression was used to select samples for Affymetrix hybridisation. qPCR fungal biomass measurements and cytological observations are available to support the gene expression data.
The wheat gene expression data available so far have shown great differences in wheat gene expression in both susceptible and resistant interactions. A far greater change was observed in wheat gene transcription during the susceptible interactions (~11,900 genes being differentially expressed) than during the resistant interactions (~3,100 differentially expressed genes). Functional analysis of the pathways up or down regulated in susceptible and resistant interactions are ongoing.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

664. Mrt, a Gene Unique to Fungi, Encodes an Oligosaccharide Transporter and Facilitates Rhizosphere Competency in *Metarhizium robertsii*.
Weiguo Fang and Raymond J. St. Leger. Department of Entomology, University of Maryland, College Park, Maryland 20742

The symbiotic associations between rhizospheric fungi and plants have enormous environmental impact. Fungi are crucial to plant health as antagonists of pathogens and herbivores and facilitate the uptake of soil nutrients. However, little is known about the plant products obtained by fungi in exchange or how they are transported through the symbiotic interface. Here, we demonstrate that sucrose and raffinose family oligosaccharides in root exudates are important for rhizosphere competence in the insect pathogen *Metarhizium robertsii* (formerly known as *Metarhizium anisopliae*). We identified mutants in the Metarhizium raffinose transporter (Mrt) gene of *M. robertsii* that grew poorly in root exudate and were greatly reduced in rhizosphere competence on grass roots. Studies on sugar uptake, including competition assays, revealed that MRT was a sucrose and galactoside transporter. Disrupting MRT resulted in greatly reduced or no growth on sucrose and galactosides but did not affect growth on monosaccharides or oligosaccharides composed entirely of glucose subunits. Consistent with this, expression of Mrt is exclusively up-regulated by galactosides and sucrose. Expressing a green fluorescent protein gene under the control of the Mrt promoter confirmed that MRT was expressed by germlings in the vicinity of grass roots but not in surrounding bulk soil. Disrupting Mrt did not reduce virulence to insects, demonstrating that Mrt is exclusively involved in M. robertsii's interactions with plants. To our knowledge, MRT is the first oligosaccharide transporter identified and characterized in a fungus and is unique to filamentous fungi, but homologous genes in Magnaporthe, Ustilago, Aspergillus, Fusarium, Epichloe, and Penicillium species indicate that oligosaccharide transport is of widespread significance.

665. RNA binding proteins mediate the ability of a fungus to adapt to the cold.
Weiguo Fang and Raymond J. St. Leger. Department of Entomology, University of Maryland, College Park, Maryland 20742

Little is known about how fungi adapt to chilling. In eubacteria, cold shock proteins (CSPs) facilitate translation by destabilizing RNA secondary structure. Animals and plants have homologous cold shock domains within proteins, and additional glycine-rich RNA binding proteins (GRPs), but their role in stress resistance is poorly understood. In this study, we identified GRP homologues in diverse fungi. However, only *Aspergillus clavatus* and *Metarhizium anisopliae* possessed cold shock domains. Both *M. anisopliae*'s small eubacteria-like CSP (CRP1) and its GRP (CRP2) homologue were induced by cold. Disrupting either Crp1 or Crp2 greatly reduced metabolism and conidial germination rates at low temperatures, and decreased tolerance to freezing. However, while both Crp1 and Crp2 reduced freezinginduced production of reactive oxygen species, only Crp1 protected cells against H2O2 and increased *M. anisopliae*'s virulence to caterpillars. Unlike CRP2, CRP1 rescued the cold-sensitive growth defects of an *Escherichia coli* CSP deletion mutant, and CRP1 also demonstrated transcription anti-termination activity, so CRP1 can regulate transcription and translation at low temperature. Expressing either Crp1 or Crp2 in yeast increased metabolism at cold temperatures and Crp1 improved tolerance to freezing. Thus besides providing a model relevant to many biological systems, Crp1 and Crp2 have potential applications in biotechnology.

666. Withdrawn

667. Gene expression analysis in sclerotia of *Sclerotinia sclerotiorum* isolates to understand the biological processes involved in sclerotial development and survival.
Vincenzo Crescente[1], Sascha Ott[2], Christophe Ladroue[3], Franz Hamberger[2], Prasad Sreenivasaprasad[1] and Dez Barbara[1]. [1]School of Life Sciences, [2]Warwick Systems Biology Centre, [3]Department of Statistics, University of Warwick, Warwickshire, UK

Sclerotinia sclerotiorum is one of the most important plant pathogens infecting more than 400 species of dicotyledonous and monocotyledonous plants worldwide. This necrotrophic homothallic pathogen produces typical structures named sclerotia, which are long-term survival structures and often the source of primary inoculum. Sclerotia can germinate myceliogenically and carpogenically; carpogenic germination leads to the production of apothecia and subsequently ascospores that infect the aerial parts of the host plants. Previous work has shown that in S. sclerotiorum isolates representing UK/European populations, exposure of sclerotia to a period of low temperature conditioning enhances their germination. On the other hand, isolates from other geographic areas may not require this conditioning to germinate. This suggests that the process is controlled by a combination of environmental and genetic factors.
We have investigated the gene expression changes in sclerotia under different conditions to understand the biological processes involved in sclerotial development and survival. To achieve this, sclerotia of two different isolate of S. sclerotiorum were used: SS-L5 from UK for which a low-temperature conditioning period is required for a good following germination; and SS-1980 from USA, for which the cold conditioning is not required. One-colour Microarray Gene Expression Analysis was performed to analyse the gene expression levels at several time points and under different temperatures. All gene expression profiles were clustered by Mathematica obtaining clusters made of genes following a similar behaviour across the experimental conditions. An Ensembl-type database containing the publicly S. sclerotiorum transcripts was generated and the transcripts of the genes included in the clusters were used to run a Blast2GO analysis to associate the gene expression changes with biological functions. A MEME motif-finding analysis in promoter regions was performed for clusters of genes with common expression pattern among the two isolates as well as clusters with isolate-specific expression. The sequence motifs identified were used to understand their potential role in the regulation of those genes.

668. Meiotic SIlencing in Neurospora. Dong Whan Lee, Robert Pratt, Ana Victoria Suescun, Ryan Millimaki, Aldrin Lugena, Alexis Brown, Michelle Yeoman and Rodolfo Aramayo. Department of Biology, Texas A&M University, College Station, TX 77843-3258

In Neurospora meiosis, if a segment of DNA is not present on the opposite homologous chromosome, the resulting "unpaired" DNA segment is targeted for silencing. This situation occurs when a DNA element gets inserted at a particular chromosomal position (e.g., a situation akin to the "invasion" of a genome by transposable DNA elements). It can also occur when a normal region gets deleted. In both situations, the resulting “loop of unpaired DNA” activates a genome-wide "alert" system that results in the silencing not only of the genes present in the "unpaired" DNA segment, but also of those same genes if present elsewhere in the genome, even if they are in the paired condition. This phenomenon is called, meiotic silencing and was originally described in Neurospora crassa, but has since been observed in nematodes and mammals. In all these organisms, "unpaired or unsynapsed" regions (or chromosomes) are targeted for gene silencing. We think that meiotic silencing is a two-step process. First meiotic trans-sensing compares the chromosomes from each parent and identifies significant differences as unpaired DNA. Second, if unpaired DNA is identified, a process called meiotic silencing silences expression of genes within the unpaired region and regions sharing sequence identity. We are using a combination of genetics, molecular biology and biochemistry aimed at identifying all the molecular players of the process and at understanding how they work together. In this work we describe the genetic, molecular, cytogenetic and biochemical characterization of key components of the system: Sms-1 to Sms-17. In addition, we describe components that are essential for the earlier stages of sexual development and discuss the connections between the vegetative pathway Quelling and Meiotic Silencing.

669. The glutathione transferase family of *Phanerochaete chrysosporium*. Mélanie Morel, Edgar Meux, Andrew Anak Ngadin, Anne Thuillier and Eric Gelhaye. UMR 1136 INRA-UHP "Interactions Arbres/Micro-Organismes", IFR110 "Ecosystèmes Forestiers, Agroressources, Bioprocédés et Alimentation", Nancy Université, Faculté des Sciences et Techniques, BP 70239, 54506 Vandoeuvre-les-Nancy, France

Glutathione-S-transferases (GSTs) form a wide family of enzymes essential in the second phase of metabolic detoxication processes. While they are well characterized in mammals and plants, GSTs have been poorly studied in fungi, despite their potential role in cell protection against antifungal and other toxic compounds. The present study concerns the white rot fungus *Phanerochaete chrysosporium*, a saprophytic basidiomycete economically promising due to its lignolytic activity. This organism was recently demonstrated to possess a large number of cytosolic GST isoforms (at least 27) distributed over six classes. Among them 2 are extended in Phanerochaete chrysosporium compared to other fungi: the Ure2p and the Omega classes. The Ure2p class possesses 9 isoforms, which cluster into 2 subclasses. Expression studies such as biochemical analysis have revealed specificities among them. Indeed, Ure2p4 and Ure2p6 are specifically expressed in condition of polycyclic aromatic compounds treatment, and Ure2p1 is atypical both because sequence divergence and enzymatic properties. The Omega class is divided in two main subclasses. PcGTO1 (subclass I, the bacterial homologues of which were recently proposed - based on their enzymatic function - to constitute a new class of GSTs named S-glutathionyl-(chloro)hydroquinone reductases (GHR)) and PcGTO3 (subclass II) have been investigated in this study. The three-dimensional structure of PcGTO1 confirms the hypothesis not only of a new biological class, but also of a new structural class that we propose to name GST xi. Indeed, it shows specific features, the most striking ones being a new dimerization mode and a catalytic site that is buried due to the presence of long loops, and which contains the catalytic cysteine.

670. Insertional Mutagenesis to Identify Novel Determinants of Pathogenicity in Rice Blast Disease. Muhammad S. Islam and Nick Talbot. Molecular plant Pathology, University of Exeter, UK

Rice blast is caused by the filamentous fungus *Magnaporthe oryzae* and is the most destructive disease of cultivated rice. The availability of the genome sequence has presented fresh challenges in terms of converting sequence data into meaningful biological information. Functional genomics studies involving the generation of large mutant collections and comprehensive screening has the potential to identify novel pathogenicity or virulence determinants. In this study, we have utilized random insertional mutagenesis to provide fresh insight into the infection mechanism of *M. oryzae* which has previously identified some novel determinants of pathogenicity in this disease (e.g. PDE1, PTH2). We have recently generated 10,000 *M. oryzae* ATMT mutants and developed a high-throughput screening system. To date, we have screened more than 8,500 mutants and confirmed a number of potential mutants either reduced or lacking in pathogenicity. It is hoped that the use of this high-throughput genetic screen will allow Identification of novel genes to develop a greater insight into the processes required for appressorium morphogenesis and more integrated understanding of appressorium mediated plant infection. A detailed understanding of the molecular basis of plant infection by *M. oryzae* will benefit the development of new strategies to control the disease and understanding the molecular basis of plant-fungus interactions.

671. A cell-type specific requirement for the C. albicans pescadillo homolog in proliferation and response to Tor inhibition. Niketa Jani, Anthony D. Hill, Folkert J. Van Werven, Robert J. Bastidas, Joseph Heitman, Julia R. Köhler. Harvard University, Cambridge, MA.

Candida albicans has evolved as a colonizer and opportunistic pathogen of mammals, and is the most successful human fungal pathogen. Among fungi infecting humans, it is unique in the frequency with which it switches between growth as budding yeast and growth as pseudohyphal and hyphal filaments. In vitro and presumably in vivo, filaments constitutively produce yeast from their subapical compartments. The C. albicans pescadillo homolog PES1 is required for this lateral yeast growth. PES1 is also required for proliferation of budding yeast cells, but hyphae continue to grow when PES1 expression from a repressible promoter is blocked. Pescadillo homologs control ribosome biogenesis and cell cycle progression, two processes tightly regulated according to nutrient availability. The Tor pathway is one of two signaling cascades that promote proliferation in response to nutrient repletion. Yeast but not hyphae require Pes1 for appropriate proliferation arrest upon Tor inhibition. When Tor signaling is inhibited by rapamycin, PES1 expression decreases dramatically and its protein product partially relocalizes. Mass spectrometry of proteins co-purified with Pes1 from yeast and hyphal cells, and from yeast exposed to rapamycin, suggests that Pes1 interacts with the replication machinery in yeast and may act as a coupling device between ribosome biogenesis and DNA replication control.

672. Light on the massive Frog decline: A Highly Sensitive Rhodopsin in *Batrachochytrium dendrobatidis.* Edgar Medina [1], Silvia Restrepo [1], and Jason E Stajich [2].[1]Departamento de Ciencias Biológicas, Universidad de Los Andes, Bogotá, Colombia.[2]Dept of Plant Pathology & Microbiology, University of California, Riverside. edmau.medina@gmail.com, srestrep@uniandes.edu.co, jason.stajich@ucr.edu

Global amphibian diversity is at great risk, and Chytridiomycosis is one of the main causes linked to this massive extinction. To develop future control strategies for this threat it is paramount to understand the molecular determinants and key elements of the life cycle that might mediate in the infection process of *Batrachochytrium dendrobatidis*(Bd). The perception of signals in critical stages of the life cycle of pathogenic fungi is strongly implicated in pathogenicity. Here we characterize with in silico tools a putative Rhodopsin (Family A of G protein-coupled receptors (GPCRs)) present in both sequenced Bd strains. Sequence and structural modeling analyses of the protein identified shared structural characteristics in the "water pocket" and the "ion lock" with ligand activated GPCRs that might lead to a higher basal activity or sensitivity to light stimuli. This identified rhodopsin is one of the first to be found in Fungi and is restricted to the Blastocladiomycota and Chytridiomycota lineages. Although GPCRs are important drug targets, understanding their importance in Fungal biology and evolution is still a challenging process due to the relatively few described, but may have important roles in lifecycle of the early diverging fungi.

673. *Mycosphaerella graminicola* succinate dehydrogenase (SDH) mutations leading to ecreased sensitivity towards SDHIs fungicides. ~~[1]Scalliet~~ G., [2]Bowler J., [2]Ward K., [1]Steinhauer D., [2]Csukai M., [1]Verras A., [1]Daina A. and [1]Fonné-Pfister R. [1] Syngenta Crop protection Münchwilen AG, Schaffhauserstrasse 215, CH4332 Stein, Switzerland. [2] Syngenta Jealotts Hill int. research center, Bracknell, Berkshire RG42 6EY, UK

Succinate dehydrogenase (SDH) is an essential enzyme of the mitochondrial tricaboxilic acid (TCA) cycle. A range of novel succinate dehydrogenase inhibitors (SDHIs) with broad spectrum against fungal pests have recently been launched into the crop protection market for the control of diseases in cereals, fruit and vegetables and seeds. In this study we explored in depth the differential binding properties of modern SDHIs to the *Mycosphaerella graminicola* enzyme using UV mutagenesis followed by selection with carboxamides of diverse structure. Molecular characterization of the four genes encoding *M. graminicola* SDH in more than 480 UV mutants led to the identification of 27 amino acid substitutions occurring at 18 different positions on 3 of the 4 subunits of the functional enzyme. A homology model of the *M. graminicola* SDH enzyme located these substitutions either within the ubiquinone binding pocket (Qp) of the SDH enzyme, or at additional positions likely to impact inhibitor binding site structure through long range effects. Mutation types and relative frequencies of occurrence were dependent on the compound and concentration used for selection. This finding was in agreement with differential resistance profiles conferred by each mutation type. Inhibitor binding properties in wild type (WT) and mutated enzyme as well as enzyme efficacy measurements were compared to *in vivo* sensitivity for a panel of 25 mutations types. This study enables us to draw some general rules explaining unexpected cross resistance profiles. Finally, full validation of this target based resistance mechanism was obtained by using homologous recombination to replace WT genes with some of the strongest and most frequent mutation types. These gene replacement strains enable us to further explore the impact of SDH mutations on pathogen fitness.

674. CYP450s role in the infection of Wheat by *Mycosphaerella graminicola* and their impact on fungicides efficacy. Na Li, Ian Southworth, Bob Dietrich, Jason Rudd and Mikaël Courbot. Syngenta Ltd, Jealott's Hill International Research Centre

The Ascomycete fungus *Mycosphaerella graminicola* (Anamorph *Septoria tritici*) causes the disease *Septoria tritici* blotch in wheat. This pathogen is typical of temperate, high-rainfall environments and is of major international importance owing to its ability to substantially reduce agricultural yields. *M. graminicola* infects both hexaploid bread wheat (*Triticum aestivum*) and tetraploid durum wheat (*T. turgidum*) and is largely controlled by fungicide applications. However *M. graminicola* has evolved resistance to several classes of fungicides highlighting the continued need to identify novel targets for disease intervention. Little is known about fungal metabolism during plant infection. As a preliminary to this project, Solexa next generation transcriptome sequence analyses were performed at different time points post inoculation of wheat leaves with the fungus as well as on liquid culture growth in which most of the fungicides are tested. The cytochrome P450 (CYP450) gene superfamily represents an ideal target for further investigation. Members of this group are (1) known to be present and regulated together with clusters of other genes important for the synthesis of secondary metabolites including mycotoxins, pigments and defence compounds and (2) known to act directly upon potentially harmful xenobiotics such as plant defence compounds and potentially fungicides in order to detoxify them. Analysis of expression of members of the CYP450 gene family has identified many which are specifically expressed early during plant infection in comparison to growth in liquid. Because of the CYP450 family size, we decided in a first step to knock-out the CPR gene and look at the effects of such mutation on fungal physiology as well as fungicides modifications. These will be further investigated initially through the generation of fungal CYP450 gene deletion/overexpression strains. It is anticipated that this project will provide new insights into the genetic basis underlying the metabolic changes occurring during *M. graminicola* infection of wheat.

675. Understanding cellobiohydrolase function by computation and experimentation. Arjun Singh[1], Larry E Taylor II[2], Gregg T Beckham[1], Todd A Vander Wall[2], Qi Xu[2], John Baker[2], William S Adney[2] and Michael E Himmel[2]. [1]National Bioenergy Center, [2]Biosciences Center, National Renewable Energy Laboratory, Golden, CO

Processive cellulases are the most well-characterized enzymes used by fungi to degrade lignocellulose in the biosphere, and are secreted at high concentrations. The most abundantly produced cellulase in most fungi are the glycoside hydrolase family 7 cellobiohydrolases (Cel7A), which are multi-domain enzymes responsible for the release of cellobiose from the reducing end of cellulose chains. To reduce the cost of enzymes in biofuels processes, we aim to first determine the fundamental molecular mechanisms of the Cel7A action on cellulose for the purpose of rational engineering of enhanced enzyme systems. We have used synthetic biology along with protein engineering supported by molecular simulation to develop a hypothesis on how Cel7A functions. Using bioinformatics tools, candidate proteins were selected based upon the estimated degree of divergence and from predicted secondary structure. The database consists of proteins from broad taxonomic, ecological, and biochemical groups. We have expressed many engineered and naturally occurring genes for Cel7A in a *Trichoderma reesei* strain from which the native *Cel7A* gene was deleted to examine the properties of the engineered Cel7A enzymes in the absence of the native Cel7A background. The wild type and mutant enzymes were analyzed using molecular dynamics simulations of interactions with crystalline cellulose to understand the molecular mechanisms responsible for observed biochemical differences between the different enzymes. We will present the results from these studies and discuss how they may be used to engineer improved enzymes of this crucial class of enzymes for cellulosic biofuels production processes.

676. Characterization of Oomycete and Fungal Effector Entry in Plant and Animal Cells. Shiv D. Kale[1], Biao Gu[1,2], Daniel G.S. Capelluto[3], Daolong Dou[1, 5], Emily Feldman[1], Amanda Rumore[1, 3], Felipe D. Arredondo[1], Regina Hanlon[1], Isabelle Fudal[4], Thierry Rouxel[4], Christopher B. Lawrence[1, 3], Weixing Shan[2], Brett M. Tyler[1] [1] Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA[2] College of Plant Protection and Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwest A & F University, Yangling, Shaanxi 712100, China[3] Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA[4] INRA-Bioger, Campus AgroParisTech, 78850 Thiverval-Grignon, France

Oomycetes cause significant damage to crops and forest each year. Analysis of several oomycete genomes shows that these genomes contain a reservoir of putative RXLR effectors. These effectors are utilized during the biotrophic phase of infection to suppress defense responses. Several oomycete and fungal effectors have been shown to enter host cells via a conserved N-terminus RxLR motif without any pathogen-encoded machinery. RxLR mediated effector entry relies on binding to a newly identified external phospholipid, PI(3)P. Binding to PI(3)P can be blocked either by sequestering PI(3)P on the outer leaflet of the plasma membrane or by competitively inhibiting the PI(3)P binding site of the effector. We present our latest findings on the mechanism of entry of oomycete and fungal RxLR effectors along with further characterization of the PI(3)P binding mechanism.

677. Microfluidic Perfusion System for Yeast Cell Microscopy. Philip Lee, Terry Gaige, Paul Hung (Presented by Julia Olsen-Claire) CellASIC Inc. Hayward, CA.

We have developed a microfluidic perfusion system for long term imaging of yeast cells in a single focal plane. The microfluidic plates are formatted to a standard 96-well plate footprint with a #1.5 thickness glass bottom to work with inverted microscopes. Yeast cells are maintained in a single focal plane during long term growth using a specially designed microfluidic chamber with a ceiling height of 4-5 microns (haploid cells) or 5-7 microns (diploid cells). The elastic ceiling holds the cells against the glass floor, keeping all cells and buds in a single focal plane for long term time-lapsed imaging. A perfusion network enables continuous feeding of the trapped cells with up to 6 different upstream solutions. Each microfluidic plate contains 4 independent flow units that are run in parallel. Flow rates in the microchannels are 1-10 ul/hr, allowing continuous imaging of over 24+ hours on the microscope stage without interruption. A custom software interface allows programmed switching of solutions during imaging to observe dynamics of cellular response. This system has been demonstrated on a variety of yeast types and strains, including *S. cerevisiae* and *S. pombe*.

678. Genome-wide analysis of the transportome of the industrial filamentous fungal strain *Penicillium chrysogenum*. Stefan Weber[1] , Jeroen G. Nijland[1], Andriy Kovalchuk[1], Remon Boer[2], Roel Bovenberg[2] and Arnold J.M. Driessen[1]. [1]University of Groningen, Department of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, and the Kluyver Centre for Genomics of Industrial Fermentation, Nijenborgh 7, 9747 AG Groningen, The Netherlands, and [2]DSM Biotechnology Center, PO Box 425, 2600 AK Delft, The Netherlands

Industrial production of β-lactam antibiotics using the filamentous fungus *Penicillium chrysogenum* is based on successive microbial strain improvement cycles. Analysis of these strains has led to the identification of several important mutations in high-producing strains. These are amongst others the amplification of the penicillin biosynthesis genes, the elevated transcription of genes involved in biosynthesis of the amino acid precursors, and genes encoding microbody proteins. However, many of the key (intra)cellular transport processes have remained obscure.
To identify ABC-transporters involved in β-lactam production, *P. chrysogenum* was grown in the presence and the absence of phenylacetic acid (PAA), a side chain precursor of penicillin G. Expression of all ABC transporters was determined by Quantitative Real Time PCR. This revealed a significantly increased expression level of ABC40 when grown with PAA. Inactivation of ABC40 in an early and a late production strain did not lead to reduced penicillin levels. However, knock-out of ABC40 did result in a higher susceptibility to phenylacetic acid, a precursor of penicillinG. More detailed analysis showed that knock-out strain of ABC40 is also more susceptible to sorbic acid and benzoic acid. Therefore we propose that ABC40 is involved in the export of weak acids amongst others phenylacetic acid.

The deathly dance of pathogens, plant hosts and their defensive mutualists. Georgiana May[1], Alma Rodriguez-Estrada[2], H. C. Kistler[3]
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Although theoretical models evaluate the evolution of pathogens with their hosts as a closed system, eukaryotic hosts are ubiquitously occupied by other microbes, such as the endophytes. Within the plant "microbiome", endophytes have been posited as defensive mutualists as they may restrain pathogen attack. In this research, we demonstrate that virulence of *Ustilago maydis* pathogen towards its maize host is correlated with the pathogen's ability to compete with a co-occurring endophytic fungus, *Fusarium verticillioides*. Theory suggests this condition should push the evolution of increasing virulence, hardly a desired result for the deployment of endophytes as biocontrol agents. Yet our empirical data points toward selection that would reinforce of a low, optimal virulence of the pathogen by the endophyte. To explain this apparent paradox, we examined the mechanisms of interaction. Our results for gene expression and metabolic analyses suggest that the endophyte most strongly restricts the growth of *U. maydis* through the production of toxins and that *U. maydis* defends itself against the endophyte, but most strongly affects the growth of the plant by causing cell death and utilizing carbon resources for its own reproduction. We suggest that interactions of these three symbionts are better represented by an intransitive rock-paper-scissors model in which each symbiont restricts most strongly the growth of one other member.

http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

A novel class of fungal lipoxygenases.

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Lipoxygenases (LOXs) are well-studied enzymes in plants and mammals. However, fungal LOXs are relatively unknown. We have compared fungal LOX amino acid sequences to all known characterized LOXs using a Shell script and the resulting sequences were aligned with MUSCLE. A phylogenetic tree was constructed to visualize the relation of fungal LOXs towards other known LOXs from all kingdoms using the protein sequences from NCBI. Fungal LOXs consist of two groups. One group has the characteristic fungal conserved WRYAK sequence and the C-terminal amino acid is isoleucine. Based on the A/G stereo specificity rule we predict this group converts polyunsaturated fatty acids to their respective *S*-stereo specific enantiomer. The other group has the highly conserved WL-L/F-AK sequence that is also found in plants and mammals. However, fungal LOXs with this sequence always have the amino acid valine at the C-terminus in contrast to other LOXs of the other group, which end with isoleucine. The overall product is predicted to be *R*-stereo specific.

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Published by New Prairie Press, 2017

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Published by New Prairie Press, 2017

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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Published by New Prairie Press, 2017

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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Published by New Prairie Press, 2017

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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http://newprairiepress.org/fgr/vol58/iss1/2
DOI: 10.4148/1941-4765.1066

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