March 15-20 2005 Asilomar Conference Center
Pacific Grove California

Fungal Genetics Newsletter, Volume 52- Supplement

XXIII FUNGAL GENETICS CONFERENCE

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Fungal Genetics Conference Program Table of Contents:

Brief Schedule

DAY	MORNING	AFTERNOON	EVENING
Tuesday, March 15		Arrival Registration	Dinner Mixer
Wednesday, March 16	Plenary Session I **GENOMES AND EVOLUTION**	Concurrent Sessions I	Poster Session I
Thursday, March 17	Plenary Session II **FUNGAL INTERACTIONS**	Concurrent Sessions II	Poster Session II
Friday, March 18	Plenary Session III **CELL BIOLOGY AND DEVELOPMENT**	Concurrent Sessions III	Poster Session III
Saturday, March 19	Plenary Session IV **SIGNALING AND GENE REGULATION**	Concurrent Sessions IV	Banquet and Invited Lecture Party
Sunday, March 20	*Ad Hoc* Sessions	Lunch Departure	

Citations

The program book for the 23rd Fungal Genetics Conference at Asilomar is published as a Supplement to the Fungal Genetics Newsletter. Please cite abstracts as follows: Fungal Genet. Newsl. 52 (Suppl):Page#
The abstracts are available online at the FGSC web-site (www.fgsc.net).

Posters

Please set your poster up in the tent outside Surf and Sand immediately after lunch the day of your poster session. Posters will be available for viewing 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.

Please thank Anne Marie Mahoney and the Genetics Society of America for their continued support of the Fungal Genetics Conference at Asilomar.

Poster Abstracts

TWENTY THIRD FUNGAL GENETICS CONFERENCE

Scientific Program

TUESDAY, March 15

3:00 pm – 10:00 pm Registration, **Administration**
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**

GENOMES AND EVOLUTION Chair: Sophien Kamoun

Rytas Vilgalys	The fungal tree of life
James Galagan	Comparative analysis of filamentous fungi
Kerry O'Donnell	The importance of species biology in a genomics era: Examples from Fusarium
Ralph Dean	The Magnaporthe grisea genome: novel insights into plant pathogenesis
Michael Snyder	Comparison of regulatory networks in yeast

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.

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

Merrill	Medical Mycology **Bill Steinbach, Joe Heitman**
Chapel	Whole Genome Comparative Analyses **Corby Kistler, Barbara Robbertse**
Fred Farr Forum	Industrial Mycology in the Age of Genomics **Linda Lasure, Peter Punt**
Kiln	Mating and Sexual Development **Bruce Miller, Robert Debuchy**
Nautilus	Regulation of Primary Metabolism **Dan Ebbole, Heather Wilkinson**
Heather	Teaching Fungal Biology and Genetics **Steve James, Patricia Pukkila**

6:00 Dinner, **Crocker Hall**
7:30 pm – 10:30 pm, **Poster Session I**

Poster Numbers	Topic
1-36	Cell Biology
137-182	Genomics and Proteomics
213-238	Industrial Biology and Biotechnology
239-280	Host Parasite Interactions
509-556	Biochemistry and Secondary Metabolism

THURSDAY, March 17

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

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

FUNGAL INTERACTIONS Chair: Joerg Kaemper

Joerg Kaemper	Regulatory networks during pathogenic development of *Ustilago maydis*
Sophien Kamoun	Extracellular protease inhibitors of *Phytophthora infestans* determine a novel counterdefense mechanism
Gillian Turgeon	Secondary metabolites and stress
Francis Martin	Genomics in the underworld: a glimpse of the interactions between ectomycorrhizal fungi and trees
Christophe D'Enfert	Post-genomic approaches to the study of biofilm formation by pathogenic Candida
Alfredo Herrera-Estrella	Prey detection and attack in an interfungal interaction

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.

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

Merrill	Fungal-Plant Interactions **Barbara Valent, Hans Van Etten**
Chapel	Population Genetics **Teresa Pawlowska, John Taylor**
Fred Farr Forum	Biological Applications of Genomic Sequence Data **Mary Anne Nelson, Melanie Cushion**
Kiln	Basidiomycete Genomics and Biology **Allen Gathman, Walt Lilly**
Nautilus	Fungal Circadian Rhythms and Photobiology **Till Roenneberg, Deb Bell-Pederson**

6:00 Dinner, **Crocker Hall**

7:30 pm – 10:30 pm, **Poster Session II**

Poster Numbers	Topic
75-99	Population and Evolutionary Biology
281-330	Host Parasite Interactions
370-410	Gene Regulation
459-508	Developmental Biology
557-588	Other

Friday, March 18

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

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

CELL BIOLOGY AND DEVELOPMENT Chair: Michelle Momany

Michelle Momany	Septins and morphogenesis in *Aspergillus nidulans*
Alex Andrianopoulos	Molecular events controlling conidial germination in *Pencillium marneffei*
Amy Gladfelter	Nuclear autonomous cell cycle regulation in multinucleated *A. gossypii* cells
Reinhard Fischer	Sex and red light in *Aspergillus nidulans*
Nick Read	Cell fusion in *Neurospora crassa*

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.

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

Merrill	Epigenetics, and Genome Dynamics **Louise Glass, Patrick Shiu**
Chapel	Secondary Metabolism **Bettina Tudzynski, Jonathan Walton**
Fred Farr Forum	Recent Advances in Oomycete Research **Jim Beynon, Pieter van West**
Kiln	Fungal Symbiotic Interactions **Natalia Requena, Arthur Schüßler**
Nautilus	The Fungal Cytoskeleton **Gero Steinberg, Robby Roberson**

6:00 Dinner, **Crocker Hall**

7:30 pm – 10:30 pm, **Poster Session III**

Poster Numbers	Topic
37-74	Cell Biology
100-136	Population and Evolutionary Biology
183-201	Genomics and Proteomics
331-369	Host Parasite Interactions
411-458	Gene Regulation
589-606	Other

SATURDAY, March 19

7:30 am - 8:30 am Breakfast, **Crocker Hall**
8:30 am – 12:00 pm Plenary Session IV, **Merrill Hall**

SIGNALING AND GENE REGULATION Chair: Katherine Borkovich

Kathy Borkovich	G protein coupled receptors control diverse environmental responses in Neurospora
Nancy Keller	Mechanistic studies of global regulation of Aspergillus secondary metabolism
Yi Liu	Molecular basis of the Neurospora circadian clock
Ben Horwitz	Signaling for disease: transducers and their target genes in the maize pathogen *Cochliobolus heterostrophus*
Jim Kronstad	Iron-regulated gene expression and signaling in *Cryptococcus neoformans*

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.

3:00 pm – 6:00 pm, **Concurrent Sessions IV**

Merrill	The Fungal Proteome and Post-Genomic Approaches to Protein Secretion **Scott Baker, Arthur Ram**
Chapel	Evolutionary Genomics **Scott Kroken, Todd Ward**
Fred Farr Forum	Cellular Morphogenesis and Development **Steve Harris, Gerhard Braus**
Kiln	Fungal Response to Stress **Paul Tudzynski, Jesus Aguirre**
Nautilus	Signal Transduction **Jin-Rong Xu, Marty Dickman**
Heather	Transporters **Uvini Gunawardena, Mike Perlin**

6:00 Banquet, **Crocker Hall**

8:00 pm – 9:00 pm Invited Lecture
Joan Bennett
Tulane University
New Orleans, LA

9:00 pm - 12:30 am Closing party, **Merrill Hall**
9:00 pm - 12:30 am Quiet alternative, **Surf and Sand Living room**

SUNDAY, March 20

8:30 am – 12:00 pm
AD HOC Workshops
Fusarium Genomics workshop
Botrytis/Sclerotinia Genomics workshop

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

12:00 pm Check-out

Concurrent Session I

3:00-6:00 pm

Medical Mycology **Bill Steinbach Joe Heitman** **Merrill**

3:00-3:25 Frank Odds, University of Aberdeen. **Population structure and virulence in *Candida albicans*.**

3:25-3:50 Aaron Mitchell, Columbia University. **From genome sequence to gene function in the fungal pathogen *Candida albicans*.**

3:50-4:15 June Kwon-Chung, NIAID/NIH. **What is the association between mating type and virulence in *Cryptococcus neoformans*?**

4:15-4:30 Tricia Missall, Saint Louis University School of Medicine. **Distinct regulation and function of the thioredoxins in *Cryptococcus neoformans* for oxidative and nitrosative stress or virulence.**

4:30-4:45 **Coffee break**

4:45-5:00 Mike Lorenz, The University of Texas Medical School. **The role of alternative carbon metabolism in *Candida*- phagocyte interactions.**

5:00-5:25 Axel Brakhage, Hans-Knoell-Institute. ***Aspergillus fumigatus* melanins and cAMP signalling: significance for virulence.**

5:25-5:45 Bill Steinbach, Duke University . **Calcineurin stress response in *Aspergillus fumigatus* pathogenesis.**

5:45-6:00 Jae-Hyuk Yu, The University of Wisconsin- Madison. **Growth and developmental mutants of *Aspergillus fumigatus*: Comparative forward genetics.**

Whole Genome Comparative Analyses **Barbara Robbertse Corby Kistler** **Chapel**

3:00-3:30 Christina Cuomo, Broad Institute. **Sequencing and analysis of the *Fusarium graminearum* genome.**

3:30-4:00 Ulrich Gueldener, MIPS. **Comparative fungal genomics using the MIPS comprehensive resources.**

4:00-4:30 Masayuki Machida, National Institute of Advanced Industrial Science and Technology. **Characterization of *Aspergillus oryzae* genome structure.**

4:30-4:45 **Coffee break**

4:45-5:10 Barbara Robbertse, Cornell University. **Identification of genes in the *Gibberella zeae* sexual reproductive pathway by cross genome comparison.**

5:10-5:35 Michael Thon, Texas A&M University. **Conservation of synteny among filamentous fungi.**

5:35- 6:00 Brett Tyler, Virginia Bioinformatics Institute. **Comparing the genomes of oomycete and fungal plant pathogens.**

3:00-3:15 Linda Lasure, Battelle Pacific Northwest National Laboratory. **Industrial Mycology in the Pre-Genomics Era.**

3:15-3:40 Noël van Peij, DSM Food Specialties. **Functional genomics in *Aspergillus niger*.**

3:40-4:05 Scott E. Baker, Pacific Northwest National Laboratory . **Fungal Proteomics and Biotechnology Research for Biobased Products.**

4:05-4:30 Mariët J. van der Werf, Department of Microbiology, TNO Quality of Life. **Metabolome analysis of *Trichoderma reesei* for the identification of metabolites linked to cellulase production.**

4:30-4:45 **Coffee break**

4:45-5:10 John C. Royer, Microbia, Inc. **Interrelationships between transcriptional, genomic and metabolic profiling data in metabolite production in *Aspergillus terreus*.**

5:10-5:25 Linda J. Johnson , AgResearch Grasslands. **Transcriptomics and metabolomics: An integrated approach to dissect endophyte-grass symbioses.**

5:25-5:40 Graham Eariss, School of Biological Sciences, Flinders University. **Directed evolution of barley beta-D-glucan endohydrolases.**

5:40-5:55 Johan van den Berg , Wageningen University. **The interplay of the pectinase spectrum of *Aspergillus niger* as revealed by DNA microarray studies.**

5:55-6:10 Arthur Ram, Leiden University. **Identification and transcriptional regulation of new starch modifying enzymes in the *Aspergillus niger* genome.**

Mating and Sexual Development **Bruce Miller** **Robert Debuchy** **Kiln**

3:00– 3:15 Bruce Miller Robert Debuchy Introductory presentation

3:15– 3:35 Bruce L. Miller, University of Idaho. **Differential regulation of fruitbody development and meiosis by the unlinked *Aspergillus nidulans* mating-type loci.**

3:35– 3:55 Carla Rydholm, Duke University. **Mating system transitions in the euascomycete genus *Aspergillus* subgenus *Fumigati* section *Fumigati*.**

3:55– 4:15 Gillian Turgeon, Cornell University. **Mating type determinants in heterothallic vs homothallic ascomycetes**.

4:15 – 4:40 **Coffee break**

Miniworkshop "Mating type evolution"

4:40 – 5:00 Xiaorong Lin, Duke University Medical Center. **Sexual reproduction between partners of the same mating-type in *Cryptococcus neoformans*.**

5:00 – 5:20 Dan Li, Texas A&M University. **A MAP Kinase pathway essential for mating and contributing to vegetative growth in *Neurospora crassa***.

5:20 – 5:40 Heather E Hallen , Michigan State University. **Differential gene expression during perithecial development in *Gibberella zeae* (anamorph *Fusarium graminearum*).**

5:40– 6:00 Thomas Fowler, University of Vermont. **Few sequence constraints hinder *Schizophyllum* pheromone function in yeast.**

Regulation of Primary Metabolism **Dan Ebbole** **Heather Wilkinson** **Nautilus**

3:05 -3:25 John Scott-Craig, Michigan State University. **Regulatory genes affecting xylose metabolism in *Cochliobolus carbonum*.**

3:25 -3:45 Chris Wong, University of Melbourne. **Post-translational regulation of AreA, the global transcriptional activator of nitrogen metabolism in *Aspergillus nidulans*.**

3:45 -4:05 Richard Oliver, Murdoch University. **Effects of primary metabolism gene disruptions on aspects of pathogenicity of *Stagonospora nodorum*.**

4:05 -4:20 Dan Ebbole, Texas A&M University. **Characterization of a sugar sensing pathway in *Neurospora crassa***

4:20-4:35 **Coffee break**

4:40– 5:00 Sven Krappmann, Georg-August University. **The Cross-Pathway Control system of *Aspergillus fumigatus* links primary metabolism to pathogenicity of this fungal pathogen.**

5:00 -5:20 Nicole Donofrio, North Carolina State University. **Gene regulation during nitrogen stress and invasive plant growth in the rice blast pathosystem.**

5:20 -5:40 Helene Tournu, Flanders Interuniversity Institute. **Nutrient sensors in *Candida albicans*: role of Gpr1 in response to glucose limitation and amino acid deprivation.**

5:40–6:00 Eurie Hong , Stanford University. **Visualization of Biochemical Pathways at the *Saccharomyces* Genome Database**

Teaching Fungal Biology and Genetics **Steve James** **Patricia Pukkila** **Heather**

3:00-3:05 Pat Pukkila and Steve James **Introductory remarks**

3:05-3:25 Pat Pukkila, University of North Carolina-Chapel Hill. **The role of faculty-graduate collaborative course design in teaching scientific reasoning to undergraduates.**

3:25-3:45 Angus Dawe, New Mexico State University, Las Cruces. **Molecular and cellular mycology: using fungal models to illustrate advanced biological concepts to students.**

3:45-4:05 Stephan Zweifel, Carleton College. **Isolating yeast mutants that affect mtDNA maintenance: a tool for teaching genetics and molecular biology to undergraduates.**

4:05-4:20 **Coffee break**

4:20-5:00 Roundtable discussion. **Emerging issues in classroom/laboratory instruction and research.**

5:00-5:20 Steve James, Gettysburg College. **Infecting an introductory bioinformatics course with your favorite fungus.**

5:20-5:40 Walt Lilly, Southeast Missouri State University. **The *Coprinus cinereus* Genome Project in the Teaching Laboratory.**

5:40-6:00 Maria Costanzo, SGD. **Visualization of biochemical pathways at the Saccharomyces Genome Database.**

Concurrent Session II

3:00– 6:00pm

Fungal Host Interactions **Barbara Valent** **Hans VanEtten** **Merrill**

3:00 – 3:05 Valent/VanEtten Introduction

3:05 – 3:15 Shun-Wen Lu, Cornell University. **Functional complementation and comparative expression analysis of CPS1, a common virulence determinant in filamentous ascomycetes.**

3:20– 3:30 Karine Lambou, CNRS / Bayer Cropscience Physiologie des Plantes et des Champignons. **Fungal tetraspanins: key players in host plant invasion.**

3:35 – 3:45 Yangrae Cho, Virginia Bioinformatics Institute. **Virulence gene discovery in the Brassica pathogen, *Alternaria brassicicola* using functional genomics.**

3:50– 4:00 Wilhelm Schaefer, University of Hamburg. **FGL1, a secreted lipase of *Fusarium graminearum* is a novel virulence factor during infection of cereals.**

4:05 – 4:15 Charlotte van der Does, University of Amsterdam, Swammerdam Institute for Life Sciences. **The *Fusarium oxysporum* avirulence protein *Six1* is required for full virulence, and is expressed early during infection of tomato.**

4:20 – 4:45 **Coffee Break**

4:45– 4:55 Yoshitaka Takano, Kyoto University. **A gene involved in modification of transfer RNA is required for fungal pathogenicity and stress tolerance of *Colletotrichum lagenarium*.**

5:00 – 5:10 Christoph Basse Max-Planck-Institute for Terrestrial Microbiology. **Mitochondrial virulence determinants in the maize pathogen *Ustilago maydis*.**

5:15 – 5:25 Anne Desjardins, National Center for Agricultural Utilization Research. **A role for ascospores in wheat head blight epidemics.**

5:30– 5:40 Regina Redman, University of Washington. **Isolation of a novel DNA sequence required for pathogenicity of *Colletotrichum* species.**

5:45– 5:55 Antonio Di Pietro, Universidad de Cordoba. ***Fusarium oxysporum* as a multihost model for dissecting fungal virulence on plants and mammals.**

Population Genetics **Teresa Pawlowska** **John Taylor** **Chapel**

3:00-3:15 Matthew Fisher, Imperial College. **Landscape genetics of *Penicillium marneffei* in southeast Asia.**

3:15-3:30 Lisa Grubisha, University of California, Berkeley. **High degree of structure is detected between populations separated by short and long distances in two sympatric *Rhizopogon* species.**

3:30-3:45 Georgiana May, University of Minnesota. **Origin and migration of *Ustilago maydis*.**

3:45-4:00 Søren Rosendahl, University of Copenhagen. **Populations, clones and individual mycelia of arbuscular mycorrhizal fungi.**

4:00-4:15 Kwangwon Lee, Cornell University. **Natural variation in the amino terminal polyglutamine domain of *Neurospora crassa* WHITE COLLAR-1 confers circadian acuity.**

4:15-4:45 **Coffee break**

4:45-5:00 Elizabeth Turner, University of California, Berkeley. **Reinforcement of reproductive isolation barriers in *Neurospora*: selective advantage and genetic basis of the early abortion of hybrid perithecia**.

5:00-5:15 Travis A. Clark, University of Toronto. **The evolutionary genetics of dikaryosis versus diploidy**.

5:15-5:30 Patrik Inderbitzin, University of British Columbia. **Horizontal transfer of selfing in the ascomycete genus *Stemphylium* (Pleosporales)**.

5:30-5:45 James Fraser, Duke University. **Convergent evolution of chromosomal sex-determining regions in the animal, plant and fungal kingdoms**.

5:45-6:00 Raymond St. Leger, University of Maryland. **Molecular adaptations for host range diversification in a fungal pathogen**.

Mary Anne Nelson , Melanie Cushion

3:00-3:20 Heather M. Hood, Oregon Health & Science University. **The *Neurospora crassa* community genome annotation project**

3:20-3:40 Anja Forche, University of Minnesota. **Demonstration of LOH by SNP microarray analysis and alterations in strain morphology in strains of *Candida albicans* during infection**.

3:40-4:00 Melanie T. Cushion, University of Cincinnati College of Medicine. **Genomic-directed development of new therapies to treat *Pneumocystis* infections**

4:00-4:20 Paul F. Morris, Bowling Green State University. **A genomic assessment of novel multi-functional protein sequences in the soybean pathogen *Phytophthora sojae***

4:20 – 4:40 **Coffee break**

4:40-5:00 Arnaud Lagorce, Bayer CropScience, La Dargoire Research Center, CNRS. **Transcriptional analysis of the pathogenic fungus *Magnaporthe grisea* during rice infection**

5:00-5:20 Elena S. Martens-Uzunova, Wageningen University. **The interplay of the pectinase spectrum of *Aspergillus niger* as revealed by DNA microarray studies**.

5:20-5:40 Hildur V. Colot, Dartmouth Medical School. **Use of yeast recombinational cloning and a *Neurospora crassa* strain defective in non-homologous end joining for high-throughput production of gene replacement mutants**

5:40-6:00 Marc Orbach, University of Arizona. **Serial Analysis of Gene Expression in *Coccidioides posadasii***.

Basidiomycete Genomics and Biology **Allen Gathman** **Walt Lilly** **Kiln**

3:00-3:20 Heather E. Hallen, MSU DOE Plant Research Laboratory. **The utility of the incomplete genome: the *Amanita bisporigera* genome project.**

3:20-3:40 Hajime Muraguchi, Akita Prefectural University. **Map construction in *Coprinus cinereus.***

3:40-4:00 Pat Pukkila, University of North Carolina, Chapel Hill. **The *Coprinus cinereus* genome project**.

4:00-4:20 Patrik Hoegger, George-August University. **The *Coprinopsis cinerea* laccase multi-gene family and evolution of multi-copper oxidase genes in basidiomycetes**.

4:20-4:35 **Coffee break**

4:35-4:55 Daniela Schubert, Friedrich Schiller University of Jena. **Involvement of ras signaling in sexual development of the basidiomycete *Schizophyllum commune***.

4:55-5:15 Paolo Amedeo, Institute for Genomic Research. **Sequencing and annotation of the genome of the human pathogenic basidiomycete *Cryptococcus neoformans* serotype D, Strain JEC21**.

5:15-5:35 Gertrude Mannhaupt, Munich Information Center for Protein Sequences. **Gene modeling and annotation of the complete *Ustilago maydis* genome.**

5:35-5:55 Barry Saville, University of Toronto at Mississauga. ***Ustilago maydis* gene expression: gene discovery to genome annotation**.

Fungal Circadian Rhythms and Photobiology **Till Roenneberg** **Deb Bell-Pedersen** **Nautilus**

3:00-3:10 Introduction

3:10-3:25 Carsten Schwerdtfeger, Dartmouth Medical School. **Alternative initiation of translation and light-specific phosphorylation yield two forms of the essential light and clock protein White Collar-2.**

3:25-3:40 Ping Cheng, University of Texas Southwestern Medical School. **Regulation of the *Neurospora* Circadian Clock by an RNA Helicase**.

3:40-3:55 Michael Brunner, Ludwig Maximilians University. **Feedback-regulation of the *Neurospora* transcription factor White Collar Complex by the circadian clock protein Frequency**.

3:55-4:10 Martha Merrow, University of Groningen. **Quantitative trait locus (QTL) analysis in *Neurospora crassa* for discovery of new circadian clock genes**.

4:10-4:30 **Coffee break**

4:30-4:45 Peter Ruoff, Stavanger University College. **A Nitrate-Induced FRQ-Less Oscillator in *Neurospora crassa*.**

4:45-5:00 Michael Vitalini, Texas A&M University. **A Genetic Selection for Circadian Output Pathway Mutations in *Neurospora crassa***.

5:00-5:15 Stuart Brody, University of California, San Diego, **Circadian Rhythms in *Neurospora crassa*: Vivid has a clock effect in constant light.**

5:15-5:30 Christian Heintzen, University of Manchester. **VVD's role in entrainment of the *Neurospora crassa* circadian clock**

5:30-5:45 Monika Schmoll, Vienna University of Technology. **ENVOY, a novel PAS/LOV domain protein, regulates cellulase gene transcription dependent on light and connects carbon source signaling to light response in *Hypocrea jecorina* (anamorph *Trichoderma reesei*)**

5:45-6:00 Round up

Concurrent Session III

3:00–6:00 pm

Epigenetics and Genome Dynamics **Louise Glass** **Patrick Shiu** **Merrill**

3:00-3:20 Patrick Shiu, University of Missouri, Columbia. **Meiotic Silencing by Unpaired DNA: localization and regulation of SAD-1**

3:20-3:40 Thomas Hammond, University of Wisconsin, Madison. **Investigating abnormal phenotypes associated with RNA silencing enzymes and RNA-dependent RNA polymerases in *Aspergillus nidulans*.**

3:40-4:00 Herman Edskes, National Institute of Diabetes and Digestive and Kidney Diseases. **What makes the *S. cerevisiae* Ure2 protein become a prion?**

4:00-4:20 Fons Debets, Wageningen University. **Het-S spore killing in *Podospora* caused by the HET-s prion**

4:20-4:40 **Coffee break**

4:40-5:00 Namboori Raju, Stanford University. **Visualization of meiotic silencing by unpaired DNA, and suppression of silencing by Spore killers in *Neurospora crassa***

5:00-5:20 Andrea Hamann, Goethe-Universität Frankfurt. **Characterization of components of an apoptotic machinery in the filamentous ascomycete *Podospora anserina*.**

5:20-5:40 Karine Dementhon, University of California, Berkeley. ***het-c* mediated heterokaryon incompatibility in *Neurospora crassa*.**

5:40-6:00 Gert Segers, University of Maryland, College Park. **The effect of viral infection on gene silencing in *Cryphonectria parasitica*.**

Secondary Metabolism **Bettina Tudzynski** **Jonathan Walton** **Chapel**

3:00- 3:20 Birgit Hoff, Ruhr-University Bochum. **The two interacting transcription factors CPCR1 and AcFKH1 control cephalosporin C biosynthesis and morphogenesis in *A. chrysogenum*.**

3:20-3:40 Motoichiro Kodama, Tottori University. **Characterization of the genomic region, controlling biosynthesis of host-specific AAL-toxins, on the conditionally dispensable chromosome of the tomato pathotype of *Alternaria alternata*.**

3:40-4:00 William C. Nierman, The Institute for Genomic Research. **Secondary metabolite biosynthetic gene clusters in filamentous fungi.**

4:00-4:20 David Keszenman-Pereyra, Krebs Institute for Biomolecular Research, University of Sheffield. **Characterization of fungal phosphopantetheinyl transferases involved in primary and secondary metabolism.**

4:20–4:40 **Coffee break**

4:40-5:00 Bettina Tudzynski, Universität Münster. **The fungal gibberellin biosynthetic pathway : gene clusters and evolution**

5:00-5:20 Barbara Howlett, University of Melbourne. **Comparative genomics of a toxin biosynthetic gene cluster in filamentous fungi.**

5:20-5:40 Frances Trail, Michigan State University. **Functional analysis of the polyketide synthase genes in *Gibberella zeae*.**

5:40-6:00 Shinichi Oide, Cornell University. **Comprehensive functional analysis of all non-ribosomal peptide synthetases in the corn pathogen *Cochliobolus heterostrophus*.**

3:00-3.30 Brett Tyler, Virginia Bioinformatics Institute. **Oomycete evolution, biology and pathology – new approaches to old questions**.

3.30-4.00 Pieter van West, University of Aberdeen. **Novel tools for the molecular characterisation of oomycete genes**.

4.00-4.10 Rays H.Y Jiang, Wageningen University. **Comparative genomics and synteny studies revealing the reservoir of secreted proteins in *Phytophthora*.**

4.10-4. 20 Wyth Marshall, University of British Columbia. **Flagellated fungal-like protists (Labyrinthulomycota and Mesomycetozoa) isolated from marine invertebrate guts.**

4. 20-4.40 **Coffee break**

4.40-5.10 Howard Judelson, University of California, Riverside. **Dissecting the spore cycle in *Phytophthora***.

5.10-5.40 Jim Beynon, University of Warwick. **Pathogenicity interactions reveal an "arms race" between *Hyaloperonosora parasitcia* and *Arabidopsis*.**

5.40-5.50 Terrence Delaney, The University of Vermont. **Identification of Arabidopsis genes that support parasitic symbiosis by *Peronospora parasitica*.**

5.50-6:00 Edgar Huitema, The Ohio State University-OARDC. **Distinct signaling pathways regulate plant cell death induced by INF1, CRN2 and PiNPP1.1 of *Phytophthora infestans*.**

3:00-3:20 Peter Lammers, New Mexico State University. **Genome sequencing of the arbuscular mycorrhizal fungus *Glomus intraradices*.**

3:20-3:40 Luisa Lanfranco, University of Torino. **Molecular mechanisms of stress response in the arbuscular mycorrhizal fungus *Gigaspora margarita*.**

3:40-4:00 Natalia Requena, University of Tübingen. **Biochemical characterization of the self-splicing protein GIN1 from the arbuscular mycorrhizal fungus *Glomus mosseae*.**

4:00-4:20 Arthur Schuessler, University of Darmstadt. ***Geosiphon pyriformis* as a model organism for the arbuscular mycorrhiza.**

4:20-4:40 **Coffee break**

4:40-5:00 Gopi Podila, University of Alabama. **Functional genomics of early interactions ectomycorrhizal symbiosis: study of Ras mediated signaling pathways.**

5:00-5:20 Tomas Johansson, University of Lund. **Global patterns of gene regulation associated with the development of ectomycorrhiza between birch and *Paxillus*.**

5:20-5:40 Ane Sesma, Sainsbury Laboratory. **Dissection of distinct pathogenesis-related processes in the blast fungus *Magnaporthe grisea*.**

5:40-6:00 Gregory Bryan, Ag. Research Ltd. **Molecular dissection of *Neotyphodium lolii*/Perennial ryegrass symbiosis**

3.00-3.05 Introduction

3.05-3.25 Berl Oakley, Ohio State University. **The roles of microtubules and actin in tip growth in *Aspergillus.***

3.25-3.40 Mouriño Pérez, CICESE, Mexico. **Microtubule dynamics during hyphal growth and branching in *Neurospora crassa***.

3.40-3.55 Günther Wöhlke, University Munich. **Role of kinesin motors in *Neurospora crassa***.

3.55-4.10 Daniel Veith, University Karlsruhe. **The role of a spindle pole body associated protein in cytoplasmic microtubule organization**.

4.10-4.25 **Coffee break**

4.25-4.45 Steve Harris, University of Nebraska. **Regulation of polarisome function in *Aspergillus nidulans***.

4.45-5.05 Gero Steinberg, MPI for terrestrial Microbiology. **Kinesin-dependent plus-end targeting of dynein is required for bidirectional endosome motility**.

5.10-5.30 Xin Xiang, University of the Health Sciences. **Cytoplasmic dynein is targeted to the spindle poles during mitosis in *Aspergillus nidulans***.

5.30-5.50 Mike Plamann, University of Missouri-Kansas City. **Genetic analysis of cytoplasmic dynein structure and function in *Neurospora crassa***.

5.50-6.05 Peter Philippsen, University Basel, Switzerland. **The Actin and Microtubule Cytoskeleton in *Ashbya gossypii***.

Concurrent Session IV

3:00–6:00 pm

The Fungal Proteome and Post-Genomic Approaches to Protein Secretion **Merrill**

Scott Baker Arthur Ram

3:00-3:15 Alistair Brown, University of Aberdeen. **Proteomic analysis of the global roles of Nrg1 in *Candida albicans*.**

3:15-3:30 Clayton Johnson, University of Arkansas for Medical Sciences. **The external proteome of *Histoplasma capsulatum*.**

3:30-3:45 Jon Magnuson, Pacific Northwest National Laboratory. **Global Proteomic Studies of *Phanerochaete chrysosporium*.**

3:45-4:00 Merja Penttilä, VTT Biotechnology. **Proteome analysis of cell responses to protein secretion in *Trichoderma reesei*.**

4:00-4:15 Thomas Guillemette, University of Nottingham. **Genomic analysis of the secretion stress response in *Aspergillus niger***

4:20-4:35 **Coffee break**

4:35-4:50 Ken Oda, National Research Institute of Brewing. **Proteome analysis of secreted proteins from *Aspergillus oryzae* in submerged and solid-state culture conditions.**

4:50-5:05 Jonathan Walton, Michigan State University. **Defining the secreteome of *Fusarium graminearum*.**

5:05-5:20 Petra Houterman, University of Amsterdam. **A proteomic approach to identify proteins secreted by *Fusarium oxysporum* in xylem sap of tomato.**

5:20-5:35 Pieter van West, University of Aberdeen. **A proteomic approach to identify extracellular and cell wall proteins involved in the *Phytophthora infestans* – plant interaction.**

5:35-5:50 Sheng-Cheng Wu, University of Georgia. **Proteomic identification and functional analysis of extra-cellular proteins from *Magnaporthe grisea*.**

Evolutionary Genomics **Scott Kroken** **Todd Ward** **Chapel**

3:00-3:20 Jason Stajich, Duke University. **Fungal intron evolution.**

3:20-3:40 Cathryn Rehmeyer, University of Kentucky. **Structure and dynamics of *Magnaporthe grisea* telomeres.**

3:40-4:00 Amy Powell, North Carolina State University. **Transposition, Recombination and Gene Genesis in *Magnaporthe***.

4:00-4:20 Betty Gilbert, University of California-Berkeley. **Comparative Genomic Hybridization within the Genus *Neurospora*.**

4:20-4:40 **Coffee break**

4:40-5:00 Jennifer Wortman, The Institute for Genomic Research. **Examination of the phylogeny and possible function of genes specific to *A. fumigatus* and *A. fischerianus.***

5:00-5:20 Brett Tyler, Virginia Bioinformatics Institute. **Genome sequences of *Phytophthora sojae* and *P. ramorum* shed new light on their evolution and pathogenicity.**

5:20-5:40 Björn Canbäck, Lund University. **An experimental screen for non-neutral evolving genes in symbiotic fungi**.

5:40-6:00 Ray St. Leger, University of Maryland. **A phylogenomic approach to reconstructing the diversification of proteases in Fungi**.

3:00-3:20 Jurgen Wendland, Friedrich-Schiller University. **Actin cytoskeletal organization is required for the maintenance of polarized hyphal growth in *Candida albicans*.**

3:20-3:40 Stephan Seiler, Georg-August University. **Analysis of hyphal morphgenesis and polar growth of *Neurospora crassa*.**

3:40-4:00 Aleksandra Virag, University of Nebraska. **Roles of *Aspergillus nidulans* Spa2 and Bud6 homologues in polarized growth and septation**.

4:00-4:10 Michael Kohli, University of Basel. **Rho GTPases, growth orientation, and the Spitzenkoper in *Ashbya*.**

4:10-4:20 Carolyn Rasmussen, University of California, Berkeley. **A Rho-type GTPase, *rho-4*, is required for septation in *Neurospora crassa***.

4:20-4:40 **Coffee break**

4:40-5:00 Bruce Miller, University of Idaho. **DopA is a Golgi complex protein that controls hyphal morphogenesis and developmental decisions in *Aspergillus nidulans***.

5:00-5:20 Gerhard Braus, Georg-August University. **Role of protein degradation in *A. nidulans* fruitbody formation.**

5:20-5:40 Stefanie Poeggeler, Ruhr-University Bochum. **Mating and fruiting body development in the homothallic ascomycete *Sordaria macrospora***.

5:40-5:00 Michael Feldbrügge, Max-Planck Institute for Terrestrial Microbiology. **Ribonucleoprotein particles that shuttle along microtubules are essential for pathogenic development in *Ustilago maydis***.

5:50-6:00 Stephen Osmani, Ohio State University. **A novel checkpoint system controlling nuclear division during *Aspergillus nidulans* mitotic exit.**

Fungal Response to Stress **Paul Tudzynski** **Jesus Aguirre** **Kiln**

3:00-3:05 Jesús Aguirre, Universidad Nacional Autónoma de México. **Introductory remarks**

3:05-3:25 Lázaro Molina, Max-Planck-Institut für terrestrische Mikrobiologie. **Characterization of a *yap1*-related gene in *Ustilago maydis***.

3:25-3:45 Jason Rudd, Rothamsted Research. **Microarray transcription profiling provides insights into metabolic regulation and stress adaptation during infection of wheat by the Septoria leaf blotch pathogen, *Mycosphaerella graminicola***.

3:45-4:05 Nora Plesofsky, University of Minnesota. **Ceramide Signals Death in the Heat-Stress Response**.

4:05-4:25 Peter Marris, University of Edinburgh. **Touch Mediated $Ca2^{+}$ signalling in *Neurospora crassa* in response to mechanical perturbation.**

4:25-4:35 **Coffee break**

4:35-4:55 Jesús Aguirre, Universidad Nacional Autónoma de México. **Reactive oxygen species regulate fungal cell differentiation.**

4:55-5:15 Paul Tudzynski, Wilhelms-Universität. **The role of ROS generating and scavenging systems in host-pathogen interaction.**

5:15-5:35 Martin Egan, University of Exeter. **Determining the role of reactive oxygen species generation in *Magnaporthe grisea*.**

5:35-5:55 Aiko Tanaka, Massey University. **Reactive oxygen species generated by a fungal NADPH oxidase regulate hyphal differentiation and growth in *Epichloe festucae*, a mutualistic symbiont of temperate grasses**

5:55-6:00 Paul Tudzynski, Wilhelms-Universität. **Summary and open questions**

Transporters **Mike Perlin** **Uvini Gunawardena** **Heather**

3:00 – 3:05 Uvini Gunawardena, Diversa Corp. **Introductory Remarks**

3:05 – 3: 25 Neil Gow, University of Aberdeen. **Cell surface mannan and the Candida-host interaction**.

3:25 – 3: 45 Markus Schrettl, Medical University Innsbruck. **Siderophore biosynthesis but not reductive iron assimilation is essential for *Aspergillus fumigatus* virulence**.

3:45 – 4:05 Wayne Versaw, Texas A&M University. **Ambient pH signaling influences phosphate acquisition in *Neurospora crassa***.

4:05 – 4:25 Barry Bowman, University of California, Santa Cruz. **The role of vacuolar calcium in hyphal morphology and tolerance of high calcium in the growth medium**.

4:25 – 4:40 **Coffee break**

4:40 – 4:50 Michael Perlin, University of Louisville. **Introductory Remarks**

4:50 – 5:10 Eric Soupene, Children's Hospital Oakland Research Institute. **The discovery of biological gas channels for NH3 and CO2: The AMT (MEP)/Rhesus superfamily**.

5:10 – 5:30 Shahram Khademi, UC San Francisco. **Ammonia channel: Structure and Function**.

5:30 – 5:50 Julian Rutherford, Duke University Medical Center. **Role of the Mep2 permease in ammonia sensing during yeast filamentous growth**

Signal Transduction **Jin-Rong Xu** **Marty Dickman** **Nautilus**

3:05-3:30 Regina Kahmann, Max Planck Institute for Terrestrial Microbiology. **The use of functional genomics for the identification of downstream genes**

3:30-3:50 Martin Dickman, University of Nebraska. **Signaling during sclerotial development in *Sclerotinia sclerotiorum*.**

3:50-4:10 Martin Gilbert, University of Exeter. **Characterisation of a P-type ATPase in *Magnaporthe grisea* defines an exocytotic pathway required for fungal pathogenicity.**

4:10-4:30 Wilhelm Hansberg, Instituto de Fisiologío Celular-UNAM. **Reactive oxygen species regulate fungal cell differentiation.**

4:30-4:40 **Coffee break**

4:40-5:00 Alexander Idnurm, Duke University. **White collar homologs sense blue light in basidiomycetes and zygomycetes: a conserved light sensing signaling cascade**

5:00-5:20 Jaehyuk Yu, University of Wisconsin. **Roles of GPCRs, G proteins and a protein kinase A in *Aspergillus nidulans*.**

5:20-5:40 Mark Caddick, The University of Liverpool. **Proteomic analysis of regulation and signalling**.

5:40-6:00 Peter S. Solomon, Murdoch University. **Dissecting the role of signal transduction in *Stagonospora nodorum* during infection on wheat.**

Fungi and the Tree of Life. R. Vilgalys, Timothy Y. James, F. Lutzoni, D. S. Hibbett, D. S. Mclaughlin, J. Sptatafora, and the AFTOL working group.

This talk will highlight recent progress in understanding evolution of the Fungal Kingdom by the NSF-sponsored project titled Assembling the Fungal Tree of Life (AFTOL). The primary aims of this study are to develop phylogenetic hypotheses using seven molecular regions (nuc-ssu rDNA, nuc-lsu rDNA, RPB2, RPB1, EF-1a , ATP6, and ITS) from approximately 1500 species representing all major groups of Fungi. The AFTOL database includes molecular and subcellular data which are continuously updated and accessible to the community via the world-wide web. Community participation is an essential component of the proposed research, which includes over 100 collaborators from at least 20 countries. A well-sampled phylogeny for Fungi will have obvious benefits for comparative genetic studies, especially with over 30 genome projects either completed or underway now for all major fungal phyla. This talk will address recent advances in identifying the earliest lineages of fungi including chytrids and zygomycetes, and their relationship to 'crown clade' fungi including basidiomycetes and ascomycetes, the origins of fungi with animals from single-celled zoosporic ancestors, as well as controversies surrounding a possible relationship between microsporidia with Fungi.
Further information about results from this project are also presented as a poster by Tim James et al. (Early diverging lineages on the fungal tree of life: phylogenetic analysis using six gene regions.)

Comparative analysis of filamentous fungi. James Galagan, Broad Institute of MIT and Harvard, Cambridge MA

Fungal genomes provide outstanding platforms for studying eukaryotic biology through comparative analysis. While sharing fundamental biology with other eukaryotes, their compact genomes allow comparative analysis techniques to be more easily developed and tested as compared to more complex organisms. We will describe the results of a comparative analysis of 3 related species of Aspergillus comprising a model organism (*A. nidulans*), a human pathogen (*A. fumigatus*), and an industrial agent (*A. oryzae*). Although members of the same genus, these species are nearly as diverged as human and fish, providing an opportunity to study genome evolution and sequence conservation over an extended evolutionary distance.

We have quantified the rate of lineage-specific rearrangements in both *A. oryzae* and *A. fumigatus*. These data provide a quantitative picture of the forces driving eukaryotic genome rearrangement, and demonstrate that rates of large and small scale eukaryotic genome evolution are not always correlated. Our analysis also led to an experimentally supported model of mating-type locus evolution that suggests the potential for sexual reproduction in both *A. oryzae* and *A. fumigatus*.

We identified numerous sequences actively conserved across the wide evolutionary distance separating the Aspergilli. By computationally enriching for statistical signatures correlated with function, we have identified several sequences with likely biological significance. These include two novel TPP riboswitches, and a conserved motif that suggests regulation by PUF family genes in filamentous fungi.

Finally, we analyzed conserved regulatory elements called upstream open reading frames (uORFs). These are open reading frames in the 5' UTRs of mRNAs in a wide range of species that have been shown to be able to regulate protein translation. We generated genomic and experimental evidence suggesting that uORFs play a larger role in regulating eukaryotic gene expression that has been previously suspected. Our analysis also provides the first genome-wide catalog of conserved uORFs in a eukaryote.

The importance of species biology in a genomics era: Examples from *Fusarium*. Kerry O'Donnell, David Starkey and Todd J. Ward. NCAUR-USDA-ARS. Peoria, IL 61604.
Fusarium head blight (FHB) poses a serious constraint to the production of wheat and barley worldwide. Infested grains are often unsuitable for food or feed due to contamination with trichothecene toxins and estrogenic compounds. In addition, FHB generally causes a significant reduction in seed quality and yields. A detailed understanding of pathogen diversity is crucial for effective disease control programs that minimize the threat of FHB. We have been investigating species limits of the etiological agents of FHB using a multilocus genealogical approach to phylogenetic species recognition. These studies indicate that the morphospecies *Fusarium graminearum* actually comprises at least thirteen phylogenetically distinct and biogeographically structured species. Remarkably, evolution of the virulence-associated trichothecene toxin genes, which are under a novel form of balancing selection, is highly discordant with the species phylogeny inferred from non-toxin cluster genes. The robust phylogenetic framework we developed is being used to investigate each species' geographic distribution, host range and toxin potential. To facilitate disease surveillance efforts, we have developed a high-throughput single tube assay for the simultaneous identification of all known B-trichothecene FHB species and their trichothecene toxin chemotypes. Review of the literature on FHB suggests that some genetic studies could benefit from the application of phylogenetic species recognition.

The *Magnaporthe grisea* genome: novel insights into plant pathogenesis. Ralph A. Dean[1], Nicholas J. Talbot[2], Daniel J. Ebbole[3], Mark L. Farman[4], Thomas Mitchell[1], Marc J. Orbach[5], Michael Thon[3], Resham Kulkarni[1], Jin-Rong Xu[6], Huaqin Pan[1], Nick D. Read[7], Yong-Hwan Lee[8], Ignazio Carbone[1], Doug Brown[1], Yeon-Yee Oh[1], Nicole Donofrio[1], Jun-Seop Jeong[1], Darren M. Soanes[2], Slavica Djonovic[3], Elena Kolomiets[3], Cathryn Rehmeyer[4], Weixi Li[4], Michael Harding[5], Soonok Kim[8], Marc-Henri Lebrun[9], Heidi Bohnert[9], Sean Coughlan[10], Jonathan Butler[11], Sarah Calvo[11], Li-Jun Ma[11], Robert Nicol[1], Seth Purcell[11], Chad Nusbaum[11], James E. Galagan[11], and Bruce W. Birren[11]. [1]Center for Integrated Fungal Research, North Carolina State University, Raleigh, NC 27695 USA. [2]School of Biological and Chemical Sciences, University of Exeter, Washington Singer Laboratories, Exeter EX4 4QG UK . [3]Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX 77843 USA. [4]Department of Plant Pathology, University of Kentucky, Lexington, KY 40546 USA. [5]Department of Plant Pathology, University of Arizona, Tucson, AZ 85721 USA. [6]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907 USA. [7]Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH93JH UK. [8]School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea. [9]FRE2579 CNRS-Bayer, Bayer Cropscience, 69263 Lyon Cedex 09 France. [10]Agilent Technologies, Wilmington, DE 19808 USA. [11]Broad Institute of MIT and Harvard, Cambridge, MA 02141 USA

Analysis of the gene set deduced from the draft sequence of *Magnaporthe* provides insight into the adaptations required by a fungus to cause disease. The genome encodes a large and diverse set of secreted proteins, including those defined by unusual carbohydrate binding domains. This fungus also possesses an expanded family of G-protein-coupled receptors, several novel virulence-associated genes, and large suites of enzymes involved in secondary metabolism. Consistent with a role in fungal pathogenesis, the expression of several of these genes is up-regulated during early stages of infection related development. The *Magnaporthe* genome has been subject to invasion and proliferation of active transposable elements, reflecting the clonal nature of this fungus imposed by widespread rice cultivation. Comparison of genome organization with other filamentous fungi reveals large blocks of common gene pairs indicative of common ancestry. The status of genome-wide gene knock-out functional studies will be presented.

Regulatory Networks in Yeast. Anthony Bormeman, Jason Ptacek, Geeta Devgan, Heng Zhu, Xiaowei Zhu, Haiyuan Yu, Paul Bertone, Joel Rozowsky, Mark Gerstein and Michael Snyder. Dept of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520

Our laboratory has been studying transcriptional and phosphorylation regulatory networks that operate in the budding yeast, *Saccharomyces cerevisiae.* We have also compared the filamentation *S. cerevisiae* transcriptional network with that of other yeasts. A summary of these results will be presented at the meeting.

Regulatory cascades during pathogenic development of *Ustilago maydis*. M. Scherer, M. Vranes, C. Pothiratana, and J. Kaemper, Max Planck Institute for terrestrial Microbiology, Marburg, Germany

In the plant pathogen *Ustilago maydis*, the change from saprophytic growth to the biotrophic stage is controlled by a unique genetic switch, namely the bE and bW homeodomain proteins encoded by the *b*-mating type locus. Our aim is to understand the processes that lead to the establishment of the biotrophic stage. To this end, we have employed genome-wide DNA arrays for *U. maydis*. The arrays were used to depict the gene expression profiles of *U. maydis* cells in response to the activation of the bE/bW heterodimer in axenic culture, but also at early and late plant infection stages. For detailed analysis of the differentially regulated genes, we have focused so far on genes encoding proteins with potential regulatory functions. By this means we were able to identify three novel pathogenicity factors for *U. maydis*. The expression profiles and phenotypes of the respective mutants revealed that they are part of a network regulating pathogenicity and filamentous growth. One of the genes, *rbf1*, encodes a transcription factor that is required (and sufficient) for the expression of the majority of *b*-regulated genes, by that assigning a central role within the network. Among the *rbf1* dependent genes are *hdp1* and *riz1*, encoding a homeobox protein and a potential zink finger transcription factor. While *hdp1* can be linked to filamentation, *riz1* is required during the early infection stages.

Extracellular protease inhibitors of *Phytophthora infestans* determine a novel counterdefense mechanism. Miaoying Tian, Jing Song, Joe Win, Nicolas Champouret, Zhenyu Liu, Sophien Kamoun. Department of Plant Pathology, The Ohio State University-OARDC, Wooster, OH.

The oomycete plant pathogen *Phytophthora infestans* secretes a diverse array of effector proteins that reprogram host processes to facilitate colonization and pathogenicity. We hypothesize that *P. infestans* secretes two classes of effectors that target distinct sites in the host plant. Effectors of the first class are secreted into the plant extracellular space (apoplastic effectors), whereas class two effectors are translocated inside the plant cell where they target distinct subcellular compartments (cytoplasmic effectors). We used data mining of genome sequences and functional genomics to identify more than 150 genes that are likely to encode secreted effectors of both classes. Our current goal is to understand the molecular function and biochemical activity of these effectors. What are their host substrates? How do they operate to reprogram host defenses?
In this presentation, we will focus on one class of apoplastic effectors that display protease inhibition activity. We used data mining of *P. infestans* sequence databases to identify 18 extracellular protease inhibitor genes, belonging to two major structural classes: (i) Kazal-like serine protease inhibitors (EPI1 to EPI14) and (ii) cystatin-like cysteine protease inhibitors (EPIC1 to EPIC4). Eight EPIs and EPICs were expressed in *Escherichia coli* and affinity purified as fusion proteins with the epitope tag FLAG. Recombinant EPI1 specifically inhibited subtilisin A among major serine proteases, and inhibited and interacted with the pathogenesis-related P69B subtilisin-like serine protease of tomato. Interestingly, EPIC1 and EPIC2 were degraded by tomato P69B but EPI1 protected both proteins from degradation. Co-immunoprecipitation experiments revealed that EPIC2 interacts with PIP1, a novel papain-like extracellular cysteine protease of tomato. Altogether, our results suggest that complex cascades of inhibition of host proteases initiated by EPI1 occur in the plant apoplast during infection of tomato by *P. infestans*. Both Kazal-like and cystatin-like inhibitors are widespread in the oomycetes, but are absent in other microbial plant pathogens. Inhibition of host proteases by *P. infestans* protease inhibitors is proposed to be a novel mechanism of pathogen suppression of plant defenses.

Secondary metabolites and stress. B. Gillian Turgeon and Shinichi Oide. Department of Plant Pathology, Cornell University, Ithaca, NY, 14853

Non-ribosomal peptide synthetases (NRPSs) are multimodular enzymes that make nonribosomal peptides (NRPs) through a thiotemplate mechanism, independent of organelles best known for protein synthesis, the ribosomes. To date, the NRPS method of peptide biosynthesis has been described only for filamentous ascomycete fungi and for bacteria. The structural diversity of possible NRP products is potentially limitless, due to flexibility in substrate choice. NRPs, polyketides (PKs) made by polyketide synthases, or NRP/PK hybrids have a broad spectrum of known biological activities, many of which have been useful in medicine, agriculture, industry, and biological research. Examples include well-known antibiotics, immunosuppressants, antitumor agents, and toxins involved in pathogenesis. However, despite the fact that activities of these small molecule secondary metabolites, with respect to interactions with other organisms, are well documented, and certainly remarkable, this cannot be their primary *raison d'etre*. In fact, the physiological significance of secondary metabolites to the producing fungi is largely unknown. Some proposed activities include roles as signal molecules for coordination of growth and differentiation (hyphal fusion, aerial hyphae formation, conidiation, sexual reproduction), as aids in the breakdown of cellular metabolic products, as defense compounds that kill competing microorganisms, as siderophores to assist in iron uptake, or as virulence effectors. We have deleted, individually, and in combination, all 12 *NPS*s encoded by *Cochliobolus heterostrophus*, the filamentous ascomycete pathogen of maize, and carried out an exhaustive phenotypic characterization of *nps*-deletion mutants to ascertain what their peptide secondary metabolite products are doing in (and for) the fungal cell. Data support our hypothesis that NRPS enzymes are purveyors of small molecules that are required for particular environmental niches, often, but not always, associated with stress conditions [e.g., low or high iron, oxidative or nitrosative stress, on the plant host, when mating, when the fungal population is too high (or too low), etc]. *Only* one is required for normal virulence of the fungus to maize, an unexpected finding, since, before this work, virulence/pathogenicity roles were among the most 'popular' suggested for NRP metabolites, at least for fungal pathogens. The remainder appear to be involved in a multitude of developmental and environment sensing processes.

Genomics in the Underworld: A Glimpse of the Interactions between Ectomycorrhizal Fungi and Trees. Francis Martin and the Laccaria Genome Consortium, UMR INRA-UHP Interactions Arbres/Micro-organismes, INRA-Nancy, 54280 Champenoux, France

The ectomycorrhizal symbiosis involving trees and soil fungi is a process of major ecological importance in temperate and boreal forests. The establishment of an effective symbiosis encompasses a series of complex and overlapping developmental processes in the colonizing mycelium and lateral roots of host trees. This includes a general growth stimulus of the rhizospheric mycelium, a trophic response directing hyphal growth inwards towards the plant tissues and morphogenetic processes leading to hyphal mantle development and intraradicular coenocytic hyphal networks. Symbiosis development also leads to novel metabolic patterns in hyphae and plant cells. In addition to morphological and physiological changes, the interaction between the ectomycorrhizal fungus and its host root is inducing a cascade of changes in gene expression in both partners. Detailed information on these molecular processes is essential for the understanding of symbiotic tissue development. We have therefore screened arrayed cDNAs to identify symbiosis-regulated (SR) genes in the ectomycorrhiza formed between Eucalyptus or Populus and their mycobionts, Pisolithus microcarpus and Laccaria bicolor. It appears that regulated gene expression is an important mechanism for controlling ectomycorrhizal symbiosis development. Major temporal patterns of induction/repression were observed with distinct groups of early-, middle-, and late-transcriptionally responsive genes to symbiosis formation. This gene profiling has permitted identification of new genes required for fungal attachment, plant defense, and symbiosis-related metabolism. These investigations showed that changes in morphology associated with mycorrhizal development were accompanied by changes in transcript patterns, but no ectomycorrhiza-specific genes were detected.
This analysis of the molecular pathways governing ectomycorrhizal symbiosis have been limited, in large part by lack of basic knowledge of fungal genomes. The U.S. DoE Joint Genome Institute and an international consortium has undertaken a shotgun approach to sequence, assemble and annote the entire 60 Mb genome of the ectomycorrhizal basidiomycete Laccaria bicolor. The current status of the genome project, including annotation, comparative and functional analyses pertaining to symbiosis, will be presented.

Post-genomic approaches to the study of biofilm formation by pathogenic Candida. C. d'Enfert[1], I. Iraqui[2], S. Goyard[1], M. Chauvel[1], S. Garcia-Sanchez[1], M. Ornatowska[1], J.M. Ghigo[3], and G. Janbon[2]. [1]Biologie et Pathogénicité Fongiques , [2] Mycologie Moléculaire, and [3]Génétique des Biofilms, Institut Pasteur, Paris, France

Candida are ubiquitous yeasts and are the major cause of life-threatening fungal infections, mainly in hospitalized hosts. We are studying the colonization of abiotic surfaces by Candida species and the formation of so-called biofilms. Candida biofilms have been found in association with catheters and prosthetic devices. Because these biofilms have a reduced susceptibility to antifungal treatments, they are an important cause of relapses after therapy.
We have taken advantage of several models of biofilm formation to investigate the transcriptome of *Candida albicans* biofilms. We have observed that diverse *C. albicans* biofilms have homogeneous transcript profiles that differ significantly from those of planktonic cultures. Inactivation of several genes over-expressed in biofilms impairs the formation of biofilms.
An alternative approach to investigate biofilm formation relied on the characterization of *Candida glabrata* insertional mutants altered in their ability to form biofilms.We have shown that a protein kinase controls biofilm formation through regulation of telomeric silencing and expression of specific adhesins. The orthologous kinase in *C. albicans* also appears as a critical regulator of biofilm formation and hyphal differentiation.

Prey detection and attack in an interfungal interaction. Alfredo Herrera-Estrella. Department of Plant Genetic Engineering. Centro de Investigación y de Estudios Avanzados del IPN. Km. 9.6 Libramiento Norte Carretera Irapuato/León. CP 36500 Irapuato, Gto. Mexico.

Filamentous fungi as heterotrophic organisms have evolved as saprotrophs and symbionts, deriving nutrients from dead organic matter or other living organisms. Most fungal species are able to establish intimate relationships with other life forms including members of their own taxa. Interfungal relationships include a variety of interactive phenomena that go from beneficial to detrimental for one of the players. The antagonism exerted by some fungi, including parasitism, on phytopathogenic fungal species has allowed us to use them as control agents in commercially important crops. Several species belonging to the genus *Trichoderma* are mycoparasites used as biocontrol agents of a wide range of economically important aerial and soil-borne plant pathogens. Conidia are used as inocula in field or greenhouse, their production is controlled by light or nutrient depletion. Unfortunately, little is known about this morphogenetic process in *Trichoderma*.
Mycoparasitism by *Trichoderma* is a complex process, including several successive steps, which may be accompanied by alternative mechanisms of antagonism. In the parasitic interaction the first detectable response is the directed growth of the hyphae of *Trichoderma* towards its pray. This phenomenon appears as a chemotropic growth in response to some stimuli in the host's hyphae or towards a gradient of chemicals produced by the host. When the mycoparasite reaches the pray, its hyphae often coil around it or attach to it by forming hook-like structures. Following these interactions, the mycoparasite penetrates the host mycelium, apparently by partially degrading its cell wall. Accordingly, it has been shown that indeed *Trichoderma* produces and secretes glucanases, chitinases, and proteases, when grown on cell walls of a pray. In fact, the *Trichoderma* genome encodes a large set of cell wall degrading enzymes. Some of the genes coding for such enzymes have been named Mycoparasitism Related Genes (MRGs), due to the clear correlation between their expression profiles and the mycoparasitic process. The development of a biomimetic system, based on the use of nylon fibers coated with a lectin purified from a host, led to suggest that these molecules played a major role in recognition. However, there is increasing evidence indicating that by-products of the host's cell wall initial degradation by *Trichoderma* enzymes trigger coiling. There is biochemical and genetic evidence for the participation of a G protein and the cAMP pathway in coiling. The induction of MRGs is independent of coiling, as a soluble signal from the host induces gene expression, even if there is no contact between the fungi. Based on the analysis of the promoter regions of MRGs, we proposed, that nutrient availability and other types of stress, could control their expression. We have demonstrated that indeed MRGs are subjected to control by nutrient availability and that a MAPK pathway determines this response. Deletion of the MAPK gene implicated in this cascade results in increased production of cell wall degrading enzymes and altered biocontrol efficacy. Interestingly, biocontrol efficacy is increased or decreased (*in vitro*) in a host dependent manner, suggesting that the same pathway may participate in host recognition. In contrast, biocontrol efficacy under gnobiotic conditions is clearly enhanced.

A septin from *A. nidulans* induces filamentous growth in *S. cerevisiae*. Rebecca Lindsey, Youngsil Ha and Michelle Momany*. Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA

Fungi grow either as tubular, multinucleate hyphae or spherical, uninucleate yeasts. Much of the machinery required for polar growth is conserved between filamentous fungi and yeasts and some fungi switch between hyphal and yeast forms in response to changes in environmental conditions. Since hyphae are found in all fungal lineages and yeasts are generally in the most derived lineages, the common ancestor of all fungi is thought to have been hyphal. *Saccharomyces cerevisiae* strains generally grow as budding yeasts. However, under some conditions diploid strains become elongated, forming filamentous pseudohyphae and some haploid strains show filamentous haploid invasive growth. Septins were first discovered in *S. cerevisiae* where they form a scaffold that organizes the bud site and are a major component of the morphogenesis checkpoint that monitors bud shape and coordinates budding with nuclear division. Five of the *S. cerevisiae* septins (Cdc3, Cdc10, Cdc11, Cdc12 and Sep7) localize to the neck region between mother and daughter cells in yeast. The other two *S. cerevisiae* septins (Spr3 and Spr28) are sporulation specific. Cdc3, Cdc11 and Spr3 localize to the leading edge of membranes during formation of the four-spored ascus. AspC, one of five septins from the filamentous fungus *Aspergillus nidulans,* induces filaments and elongated asci containing up to twelve spores in *S. cerevisiae*. AspC-induced filaments differ from previously characterized *S. cerevisiae* pseudohyphal and haploid invasive filaments. AspC-induced filaments are noninvasive and form in both haploid and diploid strains. AspC is most homologous to the Cdc12 septin of *S. cerevisiae*. GFP-AspC localizes to the necks of both filaments and buds, while GFP-Cdc12 localizes only to the necks of buds. Our results suggest that AspC competes with Cdc12 for incorporation into the septin scaffold where it induces filamentous growth.

Molecular events controlling conidial germination in *Penicillium marneffei*. Kylie J. Boyce, Sophie Zuber, Michael J. Hynes and Alex Andrianopoulos. Department of Genetics, University of Melbourne, Parkville 3010, AUSTRALIA.

Penicillium marneffei is an emerging fungal pathogen endemic to South-east Asia. In response to an extrinsic stimulus (temperature), *P. marneffei* is capable of alternating between a filamentous and a yeast growth form, a process known as dimorphic switching. *P. marneffei* grows in the filamentous form at 25˚C and in the yeast form at 37˚C. At 25˚C. Little is known about the molecular events involved in the establishment and maintenance of the various developmental states of *P. marneffei* and the control of the dimorphic switching process.
The establishment and maintenance of cell polarity is a fundamental aspect of developmental programs and cellular differentiation. Ras and Rho GTPases have been examined in a wide variety of eukaryotes and play varied and often overlapping roles in cell polarisation and development. To investigate the unique functions of these proteins and determine how they interact to co-ordinately regulate morphogenesis during growth and development we undertook a genetic analysis of GTPase function by generating double mutants of the Rho GTPases *cflA* and *cflB* and the newly isolated Ras GTPase *rasA* from *P. marneffei*. The data shows that small GTPases have both overlapping and distinct roles and genetically interact to co-ordinately regulate development.

Diving to their own drum: Nuclear asynchrony in *Ashbya gossypii* cells. Amy S. Gladfelter, A. Katrin Hungerbuehler and Peter Philippsen. Department of Molecular Microbiology, University of Basel Biozentrum, Basel, Switzerland.

Multiple nuclei residing in a common cytoplasm generally communicate and divide in unison or synchrony. In cases of DNA damage and in some multinucleated cancer cells, nuclei can behave independently or asynchronously despite sharing the same cytoplasmic environment. In the normal growth cycle, filamentous fungi such as *Neurospora crassa*, *Podospora anserina*, and *Ashbya gossypii* also have asynchronous mitoses yet the molecular basis for this nuclear autonomy remains mysterious. We are examining the controls of mitosis in *A. gossypii* with the aim of illuminating the mechanisms driving these specialized asynchronous division cycles. We have demonstrated that neighboring nuclei in *A. gossypii* cells are in different cell cycle stages despite close physical proximity and nuclear division time varies from 40 to 250 minutes, suggesting that individual nuclei independently modulate their cell cycle length. Localization and analysis of protein levels of cyclins and cell cycle regulators show that these control factors are uniformly present regardless of cell cycle stage and that protein levels do not fluctuate. Nuclei remain independent in this system even though mitotic cyclin protein transcribed from one nucleus can diffuse to and enter neighboring nuclei. Furthermore, displacing a fraction of mitotic cyclin into the cytoplasm with an exogenous nuclear export signal (NES) leads to no change in asynchrony. Thus we predict that nuclear division in this multinucleated cell is not based on oscillating protein levels as is common in eukaryotes but rather on post-translational control of cyclin dependent kinase (CDK) activity. The unique geometry of filamentous, multinucleated cells may have promoted the evolution of specialized cell cycle controls.

No sex in red light - A role for phytochromes in *Aspergillus nidulans*. Anne Blumenstein[1], Kay Vienken[1], Janina Purschwitz[1], Ronja Tasler[2], Nicole Frankenberg-Dinkel[2] and Reinhard Fischer[1]. [1]University of Marburg and University of Karlsruhe, [2]University of Braunschweig.

Phytochromes are photoreceptors that sense red and far-red light through photo-interconversion between two stable conformations. This distinct feature is mediated by a covalently bound linear tetrapyrrole chromophore. Phytochromes were thought to be confined to photosynthetic organisms including cyanobacteria, but have been recently discovered in heterotrophic bacteria and in fungi, where little is known about their functions[1]. PhsA and orthologues from other fungi share chromophore-binding regions, which are phylogenetically closer related to bacteria than to plants, pointing to their evolution from a common bacterial ancestor. In support of that, all fungal phytochromes are multifunctional proteins, where the phytochrome region and histidine kinase domain are combined in a single protein with a C-terminal response regulator domain. We studied the role of the *Aspergillus nidulans* phytochrome (PhsA). PhsA expressed in *E. coli* autoassembled with biliverdin and less efficiently with phycocyanobilin. The functionality of the two-component system histidine kinase was shown in an *in vitro* kinase assay.
We found that *A. nidulans* PhsA acts as red-light sensor and represses sexual development under red-light conditions. PhsA-GFP was excluded from nuclei, suggesting that red-light perception occurs in the cytoplasm, in contrast to blue-light perception through the nuclear flavoprotein WC-1 in *Neurospora crassa* [2,3].

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Hyphal fusion in *Neurospora*. Nick D. Read. Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JH, UK

Hyphal fusion (anastomosis) is undergone at two stages during the development of the vegetative colony of a filamentous fungus. It initially occurs between spores or spore germlings during the early stages of colony establishment, and subsequently between hyphae located behind leading hyphae of the peripheral growth zone of the mycelium. Amongst other proposed roles, vegetative hyphal fusion is believed to facilitate communication and the transport of water and nutrients within the colony by producing a complex interconnected hyphal network. *Neurospora crassa* is proving to be an excellent model system in which to analyze vegetative hyphal fusion and we are using a range of live-cell analytical techniques to study it. Much of our recent research has focused on fusion between conidia or conidial germlings by specialized hyphae, called conidial anastomosis tubes (CATs). We have shown that CATs are hyphae that are morphologically and physiologically distinct from germ tubes, and under separate genetic control. The processes of CAT induction, homing and fusion will be described, and comparisons made between CATs and mycelial fusion hyphae.

G protein coupled receptors control diverse environmental responses in *Neurospora*. Katherine Borkovich, Department of Plant Pathology, University of California, Riverside

Using reverse-genetic approaches, our laboratory has previously cloned and mutated genes encoding three G , one G and one G subunit of heterotrimeric G proteins in *Neurospora*. Analysis of the mutants demonstrated that G protein subunits regulate both asexual and sexual development in *Neurospora*. The availability of the complete *Neurospora* genome sequence has facilitated identification of at least 10 putative G protein coupled receptor (GPCR) genes in this organism. The GPCRs comprise five distinct classes, including pheromone receptors, microbial opsins, putative carbon sensors, putative nitrogen sensors and a novel group of receptor proteins not found in yeasts. Functional analysis of all 10 receptor genes is underway and results demonstrating the roles of GPCRs in sensing pheromones and nutritional status will be presented.

Metabolic Gene Cluster Silencing in *Aspergillus nidulans*. Nancy Keller[1], Keats Shwab[1], Daan Noordermeer[1], Johannes Galehr[2], Martin Tribus[2], Stefan Graessle[2], Shubha Kale[3], JinWoo Bok[1]. [1]Department of Plant Pathology, University of Wisconsin, Madison, USA 53706[2]Dept. of Molecular Biology, Medical University of Innsbruck, Innsbruck, Austria. [3] Dept. of Biology, Xavier University, New Orleans, LA, USA

In contrast to primary metabolism, the genes involved in secondary metabolism are clustered in fungi. Recently a nuclear protein, LaeA, was found to be required for the transcription of several secondary metabolite gene clusters in *Aspergillus nidulans* (Bok and Keller 2004). One of these clusters contains the positive regulatory (i.e. *aflR*) and biosynthetic genes required for biosynthesis of sterigmatocystin (ST), a carcinogenic toxin. Analysis of ST cluster expression indicates LaeA regulation of the cluster is location specific as transcription of genes bordering the ST cluster are unaffected in a *DlaeA* mutant and placement of a primary metabolic gene, *argB*, in the ST cluster resulted in *argB* silencing in the *DlaeA* background. ST cluster gene expression was remediated when an addition copy of *aflR* was placed outside of the cluster but not when placed in the cluster. Furthermore, deletion of any of three deacetylases partially remediated toxin synthesis. Site specific mutation of a S-adenosyl methionine (AdoMet)-binding site in LaeA generated a *DlaeA* phenotype suggesting the protein to be a methyltransferase. We present a model of LaeA involvement in epigenetic regulation of secondary metabolite gene clusters.

Molecular mechanism of the Neurospora circadian clock. Yi Liu, Ping Cheng, Qun He, Qiyang He, and Yuhong Yang Department of Physiology, UT Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9040

FREQUENCY (FRQ), WHITE COLLAR-1 (WC-1) and WC-2 proteins are three critical components forming the circadian negative feedback loop in Neurospora. We recently identified FRH, a FRQ-interacting RNA helicase, as another essential component of the circadian negative feedback loop. Down-regulation of FRH by dsRNA leads to the abolishment of circadian rhythms and impaired function of the negative feedback loop. FRH recruits FRQ to the WC complex and together FRQ, they function as the negative element of the negative feedback loop.

FRQ is progressively phosphorylated and its level decreases when it is extensively phosphorylated. FRQ phosphorylation is regulated by several kinases, including CKI, CKII and CAMK-1. Our data showed that CKII is an essential clock components, and that the phosphorylation of FRQ by CKII promotes FRQ degradation and is important for the closing of the circadian negative feedback loop. On the hand, two protein phosphatases, PP1 and PP2A, play distinct roles in the Neurospora clock:
PP1 regulating FRQ stability while PP2A is important for the function of the circadian feedback loop. After FRQ is phosphorylated, it is degraded through the ubiquitin-proteasome pathway. Such degradation is mediated by FWD-1 (an F-box/WD-40 repeat-containing protein), which is the Neurospora homolog of the Drosophila Slimb protein.
In addition to the critical role of WC-1 and WC-2 in the circadian feedback loop, both proteins are essential components for the light input of various blue light responses, including the light entrainment of the circadian clock. We showed that the putative flavin-binding domain of WC-1, its LOV domain, is required for light responses. By purifying the endogenous WC complex from Neurospora, we showed that it is associated with FAD, suggesting that WC-1and other LOV domain containing proteins are blue light photoreceptor mediating light responses in Neurospora.

Signalling for disease: transducers and their target genes in the maize pathogen *Cochliobolus heterostrophus*.
Benjamin A. Horwitz, Department of Biology, Technion, Haifa, Israel

Conserved eukaryotic signaling pathways play important roles in the development of fungal pathogens. In the maize pathogen *C. heterostrophus*, targeted disruption of genes for heterotrimeric G protein subunits and a MAP kinase has shown that the pathways they belong to are needed for various aspects of morphogenesis, virulence, mating and sporulation. Loss of the G protein alpha subunit gene *CGA1* results in altered hyphal growth and female sterility, yet virulence is not lost, rather altered as a function of host physiology. Loss of the G protein beta subunit gene *CGB1* or of MAP kinase *CHK1* drastically reduces virulence. By suppression subtractive hybridization, we identified targets of the *CHK1* MAPK pathway, characterizing genes for two cellulolytic enzymes and two of unknown function. Some transcription factors detect signals without the intermediary of classical signal transducers. A redox sensitive transcription factor, ChAP1, is an ortholog of yeast YAP1, and regulates several anti-oxidant genes. ChAP1 relocalizes to the nucleus and induces target genes in response to oxidative stress and plant factors. We are now studying the interrelation of signal transduction pathways and direct detection by transcription factors in the response of *C. heterostrophus* to light, stress and host plant factors.
Lev & Horwitz (2003) Plant Cell 15:835-844; Ganem et al. (2004) EC 3:1653-1663; Degani et al. (2004) AEM 70: 5005-5009; Lev et al. (2005) EC in press; M. Giloh, S. Lev, R. Hadar: in progress.

Iron-regulated gene expression and signaling in *Cryptococcus neoformans*. Jim Kronstad, Tianshun Lian, Megan Simmer, Barbara Steen, Guanggan Hu, and Cletus D'Souza
The Michael Smith Laboratories, University of British Columbia, Vancouver, B.C., Canada

The basidiomycete fungus *Cryptococcus neoformans* is an opportunistic pathogen of immunocompromised hosts. Recently, a related species, *C. gattii*, has emerged as a primary pathogen as demonstrated by an outbreak of infections on Vancouver Island. Genomic resources have been developed by the Cryptococcus research community and these include genome sequences for three strains of *C. neoformans* and two strains of *C. gattii*. We have used these resources and serial analysis of gene expression (SAGE) to analyze RNA expression in response to temperature, disruption of cAMP signaling and iron deprivation. We are particularly interested in iron limitation because this condition enhances production of the polysaccharide capsule that is the major virulence factor of the fungus. Sets of iron-responsive genes were identified by constructing and analyzing SAGE libraries for strains of two capsular serotypes of *C. neoformans* after growth in low-iron and iron-replete media. In addition to a comparative analysis of the SAGE data, we have focused subsequent work on characterizing the roles of the *FTR1* and *CIG1* genes encoding an iron permease and a cytokine-inducing glycoprotein, respectively. Disruption of *FTR1* indicates that iron uptake is important for the control of capsule size and disruption of *CIG1* reveals a role in sensing iron levels.

Poster Session Abstracts

Cell Biology

1. Evaluation of a recombinant pH-sensor in *Aspergillus niger* K Altenbach[1], T Bagar[2], ND Read[1], M Bencina[2]. [1]Fungal Cell Biology Group, Institute for Cell Biology, University of Edinburgh, Edinburgh EH9 3JH, UK [2]Laboratory of Biotechnology, National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

We have developed a recombinant pH-probe allowing a novel approach to pH-measurement in filamentous fungi. It is known that the absorbance and fluorescence of some GFP mutants depend strongly on the pH. Based on pHluorins, we have designed a novel variant of GFP, called RaVe_C, and expressed it in *Aspergillus niger*. RaVe_C is a ratiometric dual excitation pH-sensor displaying reversible emission ratio changes in the range of pH 5.5 to pH 7.5. With a pK_a of 7.0 RaVe_C is ideal for monitoring pH-changes in the cytosol. We have analysed the performance of this probe in *A. niger* using confocal laser scanning microscopy, addressing the following questions: (1) Is the level of expression of RaVe_C in *A. niger* high enough for imaging? (2) Does the probe respond to changes in intracellular and extracellular pH? (3) How can we best calibrate the pH readout of RaVe_C expressed in *A. niger*?

2. Genetic analysis of cytoplasmic dynein structure and function in *Neurospora crassa*. Michael Plamann, David Madole, Dipti Gandhe, and Sonali Joshi. University of Missouri-Kansas City, School of Biological Sciences, Division of Cell Biology and Biophysics, Kansas City, MO 64110

Cytoplasmic dynein is a large, microtubule-associated motor complex that facilitates minus-end-directed transport of various cargoes. Dynein heavy chain (DHC) is >4000 residues in length, with the last two-thirds of the heavy chain forming the motor head. Six domains within the dynein motor exhibit varying degrees of homology to the AAA+ superfamily of ATPases. These domains are followed by a distinct C-terminal domain and together form a ring-like structure from which a microtubule-binding domain protrudes. Using a genetic assay in the filamentous fungus *Neurospora crassa*, we have isolated over 50 DHC mutants that produce full-length protein, but are defective in function. We have identified DHC point mutations in various areas including 1) AAA#1, the AAA module known to bind and hydrolyze ATP; 2) AAA#3, an AAA domain thought to regulate microtubule binding; 3) AAA#6, a domain with degenerate AAA homology lacking the conserved Walker boxes; and 4) AAA"#0", a globular domain with feint AAA homology which is N-terminal to AAA#1. In filamentous fungi such as *N. crassa*, cytoplasmic dynein is required for nuclear migration and retrograde vesicle transport of organelles. While all DHC point mutations examined so far are deficient in vesicle transport, some DHC point mutants exhibit apparently normal nuclear distribution phenotypes.

3. The role of RacA and the identification of RacA interacting proteins during polarized cell growth in *Aspergillus niger*. M. Arentshorst[1], R.A. Damveld[1], S.M.J. Langeveld[1], M.S. Roelofs[1], C.A.M.J.J. van den Hondel[1,2] and A.F.J. Ram[1,2]. [1]Leiden University, Institute of Biology Leiden, Fungal Genetics Research Group, Leiden, The Netherlands, [2]Department of Microbiology, TNO Nutrition and Food Research, Zeist, The Netherlands.

The establishment of cell polarity in filamentous fungi is critical for the control of many cellular and developmental processes, including polarized hyphal growth, intracellular movement of organelles, protein secretion, cell wall biosynthesis and the development of conidiophores. In yeasts, but also in other eukaryotic cells, polarized cell growth is driven by Rho-related GTPases (Rho, Rac and Cdc42). Analysis of the function of the small GTPase RacA in *A.niger* has implicated a role for RacA during polarized cell growth. Deletion of *racA* showed abnormal branching at the hyphal tip and delayed sporulation. Overexpression of dominant active RacA resulted in the loss of polarized hyphal growth. To identify RacA interacting proteins Gateway compatible yeast two hybrid cDNA libraries have been constructed. The effects of alteration of expression of these RacA interacting proteins on fungal morphology will be determined.

4. Isolation and characterization of *NcSKN7* and *NcOCH1* disruptants in *Neurospora crassa*. Shinpei Banno[1], Makoto Kimura[2], Isamu Yamaguchi[2, 3], Makoto Fujimura[1]. [1]Dept of Life Sciences, Toyo Univ, Gunma, Japan. [2]Laboratory for Remediation Research, PSC, RIKEN, Wako, Japan. [3]Laboratory for Adaptation and Resistance, PSC, RIKEN, Yokohama, Japan
Two-component signal transduction pathway consisting of Os-1p (histidine kinase), Rrg-1p (response regulator) and Os-4p, 5p, 2p (MAP Kinase cascade) plays an important part in osmotic adaptation and fungicide-resistance of *Neurospora crassa*. Like yeast, *N. crassa* has two response regulators, Rrg-1p and NcSkn7p. To investigate the function of NcSkn7p branch in histidine kinase pathway, we isolated and characterized the gene disruptants of *NcSKN7* and also of *NcOCH1*. NcSkn7p has HSF-like (heat shock factor) binding domain and response regulator domain. The *NcSKN7* gene disruptant by gene replacement didn't affect osmotic sensitivity and fungicide resistance, therefore the NcSkn7p branch might not be directly involved in osmoregulation. The growth and fertility of *ncskn7* disruptant were normal, but it was slightly sensitive to oxidative stresses (H_2O_2 and t-butyl hydroperoxide). The insertional disruptant of *NcOCH1* gene, which encodes a putative alpha-1, 6-mannosyltransferase, showed slow growth and severe morphological abnormality; coral-like multiple branching hyphae with few conidia. We analyzed electrophoretic mobility of a glycoprotein, acid phosphatase. The *ncoch1* disruptant cells produced a smear band with difference mobility by activity staining, suggesting that the function of *NcOCH1* was involved in outer chain elongation of oligosaccharides.

5. Identification of Antibiotic Binding Sites in the V-ATPase. Emma Jean Bowman, Marija Draskovic, Molly McCall, and Barry Bowman. Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, CA 95064, USA

The macrolide antibiotics bafilomycin and concanamycin are potent inhibitors of V- ATPases. To identify the binding site of bafilomycin we selected mutant strains of *Neurospora crassa* (named *bfr*) that are resistant to this antibiotic. In one class of bfr strains the V-ATPase was resistant to inhibition *in vitro*. These strains had seven different point mutations in the *vma-3* gene, which encodes the hydrophobic c subunit of the vacuolar ATPase. Most of the mutated sites appear to be on the outer face of the "rotor" sector of the enzyme, a region hypothesized to form an interface with the "a" subunit.
Surprisingly, the *bfr* strains had little resistance to concanamycin, which has a similar structure. By further mutagenizing three *bfr* strains we obtained 13 new strains that were resistant to both antibiotics. Each of these had two altered residues in the c subunit. Thus, concanamycin does appear to bind to the same region. The positions of four of the mutated residues correspond precisely to the positions of mutated residues in the homologous c subunit of the mitochondrial ATPase that confer resistance to oligomycin. These results suggest that vacuolar and mitochondrial ATPases have an ancient, conserved antibiotic binding site. As the sequences of the polypeptides have diverged new antibiotics that target the same vulnerable site in this family of enzymes have arisen. The data also suggest a model for the tertiary structure of the c subunit of the V-ATPase. To test the model we have developed new procedures for introducing mutations into subunits of the V-ATPase.

6. The role of vacuolar calcium in hyphal morphology and tolerance of high calcium in the growth medium. Barry Bowman, Marija Draskovic, Emilio Margolles-Clark, and Stephen Abreu. Department of Cell, Molecular and Developmental Biology, University of California, Santa Cruz, CA 95064

Calcium, a key signaling molecule in all organisms, has been postulated to play a major role in polar hyphal growth in filamentous fungi. However, we know little about which organelles or transporters are involved in sequestration of calcium within hyphae. Analysis of the *Neurospora crassa* genome revealed several calcium transport proteins that may function in organelles (Zelter et al. FGB 41:2004). The *cax* gene encodes a proton/calcium exchange protein, homologous to the Vcx1/Hum1 protein in the vacuole of *S. cerevisiae*. *Nca-2* and *nca-3* encode P-type Ca-ATPases homologous to the Pmc protein also reported to be in the vacuole of *S. cerevisiae*. We generated mutant strains lacking these transporters and used cell fractionation to examine the distribution of calcium. In the wild-type strain only the vacuolar fraction had significant amounts of calcium. The concentration in this organelle was surprisingly high, at least 20 mM. *Nca-2* and *nca-3* mutant strains were like wild type, but inactivation of the *cax* gene caused the complete loss of calcium from the vacuole. When strains were inoculated in medium with high concentrations of calcium (50-200 mM) wild-type, *nca-3* and *cax* strains grew normally, but growth of the *nca-2* mutant and of the *nca-2 cax* double mutant was strongly inhibited. In standard growth medium (Vogel's, 1 mM calcium) all the mutant strains had normal hyphal morphology. Thus, the results indicate that the vacuole is a major storage site for calcium; however, loss of vacuolar calcium does not affect polar growth or the ability to tolerate high concentrations of calcium in the medium.

7. Circadian Rhythms in *Neurospora crassa*: Clock Mutant Effects in the Absence of a frq–based Oscillator. Laura Lombardi, Kevin Schneider, Michelle Tsukamoto, and Stuart Brody
Div. of Biological Sciences, UCSD, La Jolla, CA 92093-0116

In Neurospora, the circadian rhythm is expressed as rhythmic conidiation due to a feedback loop involving the genes and protein products of frq (frequency), wc-1 (white collar-1), and wc-2, known as the frq/wc (FWC) oscillator. Although null mutations, such as frq10 or wc-2(delta) that lack a functional FWC oscillator, lead to the loss of this rhythm under most conditions, a rhythm can be restored to them by the addition of geraniol or farnesol to the media. Utilizing this restoration as an assay, the effect of other clock mutations in a frq10 or wc-2(delta) null background can then be measured. It was found that existing clock mutants fall into 3 classes: 1) those that showed NO effect in a null background, such as prd-3 frq10 and frq7 wc-2(delta) (proof of principle); 2) those that did have a measurable effect in the frq10 background, such as prd-1, or prd-4; and, 3) those that suppressed the frq10 effect, such as ult-1, i.e. geraniol/farnesol were not required for a visible rhythm. Using analysis of these results along with previous studies, one can classify these double mutants into 3 categories:
I . Both mutations affect the FWC oscillator, such as prd-3 or frq10 or wc-2(delta);
II. Both mutations do not affect the FWC oscillator, such as prd-4, prd-1, cel, chol or ult;
III. One mutation affects the FWC, one does not, such as frq10 or prd-4.
Classifying which clock mutations affect the FWC oscillator and which do not is a first step to understanding a multi-oscillator system.

8. Circadian Rhythms in *Neurospora crassa*: Vivid has a clock effect in constant light. Stuart Brody and Michelle Shuff, Division of Biological Sciences, UCSD, La Jolla, CA.

Previous studies on vivid (vvd) mutants have shown that vivid over-produces carotenoids in the light, is involved in photoadaptation, and that the bd vvd strain has a normal period in constant darkness (D/D) of 23 hrs., but no observable rhythm in constant light (L/L) under the conditions of race tubes, glucose-arginine media (Shrode et al. , Fungal Genet.Biol., 32,169 (2001), Schwerdtfeger and Linden, Mol. Microbiol., 39, 1080 (2001), Heintzen et al., Cell,104, 453 (2001)). Our studies showed the following: 1) the above-mentioned findings were reproducible; 2) however our use of different media/conditions (maltose/Petri plates) for the bd vvd strain lead to short (7-10 hr.) periods in L/L (fluorescent); 3) the period in D/D was still 23 hrs. ; 4) the bd control strain showed no rhythm in L/L; 5) a bd strain containing the vvd allele (ss) also showed 8-10 hr periods in L/L; 6) the introduction of the csp-1 marker (conidial separation) and the use of a different media, namely acetate/casamino acids, gave the clearest banding, averaging 8-11 hr. periods; 7) the periodicity of the bd csp-1 vvd strains are a function of the intensity of the light; and 8) the rhythm in L/L is temperature-compensated. These findings suggest that the vvd gene plays, under certain conditions, a more significant role in clock timing than previously suggested.

9. Cdk2, a second essential cyclin-dependent kinase in the corn smut fungus *Ustilago maydis.* Sonia Castillo-Lluva and José Pérez-Martín. Centro Nacional de Biotecnología-CSIC. Campus de Cantoblanco-UAM. 28049 Madrid. Spaain

Cdk2 is a member of the Pho85 family of cyclin-dependent kinases. Unlike other fungal members of this family, Cdk2 appears to be essential for growth in U. maydis. A temperature-sensitive allele of cdk2 was generated, caused cell separation defects, G1 arrest and polarity defects at restrictive temperature. Therefore, the essential function may involve cell cycle control and morphogenesis. To further characterize the roles of Cdk2 in U. maydis, we also analyzed the putative cyclin partners. We found five distinct cyclin genes (pcl1-5) and we started the search for suppressors of the growth defects present in the ts-mutant. Details of this search will be provided.

10. Cytoplasmic flow in *Neurospora crassa* during the circadian cycle. E. Castro-Longoria[1], S. Brody[2], and S. Bartnicki-García[1]. [1]División de Biología Experimental y Aplicada, CICESE, Ensenada, B. C., México. [2]Division of Biology, University of California, San Diego.

The radial growth rate of a colony of *Neurospora crassa* (*bd csp oli* strain) undergoes circadian oscillation with a reduction of approximately 42%, coincident with the conidiation process. Conidium-free (interbands) and conidia-laden bands are formed alternatively in each 24h cycle. Since hyphal growth depends on precursors mobilized over long distances, we investigated if the changes in growth rate were correlated with changes in cytoplasmic flow. The flow of cytoplasm in vegetative hyphae was examined at high magnification. At the growing edge of the colony, cytoplasm was found to flow continuously towards the leading hyphae and their branches during formation of either band. Further back, at about 500 m from the edge of the colony, cytoplasm from older branches was observed to drain into leader hyphae, thus contributing to the formation of the growth front. Leader hyphae and branches in the interband, just behind a newly formed conidial band, became progressively vacuolated and the amount of cytoplasm decreased in older sections until a high percentage of hyphae were completely empty. Clearly, the cytoplasm of the interband hyphae is mostly diverted away from the forward extension of the colony towards the formation of aerial hyphae and the subsequent production of conidia. Presumably, the observed circadian decrease in vegetative growth rate is a consequence of the increased demands of cytoplasm to support the conidiation phase.

11. Biochemical analysis of Dbf4-dependent kinase in *Aspergillus nidulans*. Bernadette Connors, Jennifer Line, Morgan Campbell, and Steven James. Department of Biology, Gettysburg College, Gettysburg. PA 17325

In the fungus *Aspergillus nidulans*, *nimO* and *cdc*7 encode the regulatory and catalytic subunits of the conserved eukaryotic Dbf4-dependent kinase (DDK). In budding yeast, dbf4p escorts cdc7p to origins of replication, and origin unwinding is triggered through phosphorylation of pre-RC components by cdc7p. Dbf4p is subsequently targeted for destruction by the anaphase promoting complex through D-box motifs found in its amino terminus, thus preventing reinitiation of DNA synthesis. In *Aspergillus*, the temperature sensitive lethality of the *nimO*18 mutation can be partially suppressed by mutations in two genes, *snoA* and *snoB* (suppressor of *nimO*). As one approach to investigate DDK function we generated epitope tagged alleles of both *cdc*7 (*HA*3::*cdc*7) and *nimO* (*HM*2::*nimO*). Standard immunoblotting techniques have shown that HA3::cdc7p is a phosphoprotein that is differentially phosphorylated through the cell cycle. Preliminary results indicate this phosphorylation occurs in either a *nimO*- or *snoA*-dependent manner and current efforts include a detailed examination of HA3::cdc7p in both *nimO*18 and *snoA* mutant backgrounds. HM2::nimOp is also a phosphoprotein and our current efforts include examining nimOp turnover and phosphorylation through the cell cycle in strains that harbor mutations in the two N-terminal D-boxes. Supported by NSF-RUI #01-14446 to SJ.

12. A novel checkpoint system controlling nuclear division during *Aspergillus nidulans* mitotic exit. Jonathan Davies, Colin De Souza and Stephen Osmani. Department of Molecular Genetics, Ohio State University, Columbus, OH 43210

In order to generate multinucleate cells, filamentous fungi, including *Aspergillus nidulans*, undergo rounds of nuclear division in the absence of cytokinesis. During these closed mitoses, nuclear division must be driven by forces other than cytokinesis. In contrast, it has been suggested that cytokinesis drives nuclear division in budding yeast by promoting nuclear fission.
In yeasts a "dumbbell" shaped nucleus can be observed during nuclear division. In contrast, we have failed to detect "dumbbell" shaped nuclei during mitotic exit in *A. nidulans*. Therefore, nuclear fission, during which constriction of the nuclear envelope generates two nuclei from one, may not occur in *A. nidulans*. However, nuclear division is a regulated process. For instance, dominant versions of *tinD* promote defective mitoses in which anaphase proceeds but, surprisingly, nuclear division does not occur. The result of this defective mitosis is an intact polyploid nucleus which can progress through further cell cycles.
We propose that a regulatory mechanism exists in *A. nidulans* which, following detection of mitotic defects, allows completion of DNA segregation but stops nuclear division during mitotic exit. Because mitosis is not followed by cytokinesis, the resulting nucleus is able to contribute to hyphal growth. Through the process of haploidization, normal haploid nuclei can also be subsequently generated. Thus, filamentous fungi can prevent inappropriate segregation of DNA to daughter nuclei after defective mitosis by preventing nuclear division. This survival strategy is not available to unicellular organisms, such as yeasts, because nuclear division is linked to cytokinesis.

13. Mitotic Regulation of the Nuclear Pore Complex in *Aspergillus nidulans*. Colin De Souza, Shahr Hashmi, Aysha Osmani and Stephen Osmani. Department of Molecular Genetics, Ohio State University, 802 Riffe Building, 496 W 12th Ave Columbus OH 43210.

During *Aspergillus nidulans* closed mitosis, the nuclear pore complex (NPC) is partially disassembled, facilitating nuclear entry of tubulin and mitotic regulators (De Souza et al. 2004. Current Biology 14:1973-84). Genetic, overexpression and localization studies all implicate the NIMA mitotic kinase in regulating partial NPC disassembly during mitosis. We hypothesize that NIMA triggers the mitotic dispersal of the SONA and SONBn NPC proteins (nucleoporins) from the NPC as the first step in mitotic NPC disassembly. The $SONBc^{Nup96}$ structural nucleoporin remains associated with the nuclear envelope throughout mitosis. SONA and SONBn bind each other and are likely tethered to the NPC by binding of SONBn to $SONBc^{Nup96}$.
To define how the initial steps in mitotic NPC disassembly are regulated, we have determined the minimal domain within SONBn required for binding to the NPC. We demonstrate that this domain ($SONBn^{587\text{-}1035}$) disperses from the NPC during mitosis and is mitotically phosphorylated in a NIMA-dependent manner. $SONBn^{587\text{-}1035}$ contains 8 consensus NIMA phosphorylation sites and we demonstrate that mutation of all eight sites to glutamic acid compromises binding to the NPC. To determine if these sites (or other sites) are phosphorylated in vivo, we have generated an S-tagged version of $SONBn^{587\text{-}1035}$ allowing single step purification using S-protein agarose. S-tag-$SONBn^{587\text{-}1035}$ has been purified to homogeneity from mitotic *A. nidulans* extracts to facilitate the identification of phosphorylated residues utilizing mass spectrometry. Similar strategies are being employed to determine the sites of mitotic phosphorylation in $SONBc^{Nup96}$.

14. Functional analysis of ABC transporter genes in *Aspergillus nidulans*. Maarten A. De Waard[1], Ciska Braam[1], Lute-Harm Zwiers[1], and Alan C. Andrade[2]. [1]Laboratory of Phytopathology, Wageningen University, The Netherlands. [2]Embrapa Recursos Genéticos e Biotecnologia, Brasilia-DF, Brazil.

At present, seven ATP-binding cassette (ABC) transporter genes (*atrA*-*atrG*) from *Aspergillus nidulans* are functionally characterized. Previously, *atrB* and *atrD* were identified as multidrug transporter and transporter of antibiotics, respectively. Recently, *atrG* was characterized as a transporter of azole antifungals. Its physiological function may involve transport of sterols, since sterols induce expression of *atrG*, reduce efflux of azoles from intact cells, and affect growth of *atrG* knockout mutants in a temperature-dependent manner. A triple knockout mutant of *Aspergillus nidulans* of *atrB*, *atrD*, and *atrG* is hypersensitive to a wide range of natural toxic products and drugs and its phenotype is the combination of that of the single knockout mutants. The triple knockout mutant can be useful in screening programs to detect new antifungals. The *imaB* mutation, previously described in relation to pleiotropic drug resistance, enhances transcription of *atrD*, *atrE*, *atrF*, and *atrG*, suggesting that *imaB* may be a regulatory gene of ABC transporter genes. Currently, we try to clone *imaB* by complementation of *imaB* mutants with a gene-specific cosmid library.

15. *het-c* mediated heterokaryon incompatibility in *Neurospora crassa*. Karine Dementhon, Qijun Xiang, Isao Kaneko and N. Louise Glass. Plant and Microbial Biology department, UC Berkeley, California, 94720

Filamentous fungi are capable of undergoing hyphal fusion with each other to form a vegetative heterokaryon (genetically different nuclei in a common cytoplasm). However, if individuals undergoing hyphal fusion differ in allelic specificity at any one of a number of heterokaryon incompatibility loci called *het*, the heterokaryotic cell is rapidly compartmentalized and destroyed by a programmed cell death reaction. This phenomenon is called heterokaryon incompatibility. This nonself recognition is believed to reduce the risk of transmission of infectious elements such as mycoviruses and debilitated organelles throughout fungal populations, and to restrict resource plundering between individuals.

In *Neurospora crassa*, incompatible heterokaryons show growth inhibition, repressed conidiation and hyphal compartmentation and death. Three antagonist *het-C* alleles are present in populations: *het-C*^{OR}, *het-C*^{PA} and *het-C*^{GR}. HET-C is a plasma membrane protein. *het-c* incompatibility is suppressed by *vib-1*, which encodes a putative nuclear- localized transcriptional regulator. Mutations in *vib-1* result in strains showing de-repressed conidiation. Here we show that *vib-1* over-expression represses conidiation, confirming that VIB-1 is a repressor of conidiation. We show that VIB-1 is a nuclear-localized protein by constructing a GFP fusion protein. VIB-1 is detectable when expressed under its native promotor *in vivo*. During incompatibility, VIB-1-GFP is also detected in nuclei, with an increased intensity of fluorescence.

Another locus, *vib-2*, mapped near *het-c*, is required for *het-c* incompatibility. *vib-2* is predicted to encode a HET domain protein. *het-c/vib-2* non-allelic interactions are essential for *het-c* incompatibility. *vib-2*^{OR} and *vib-2*^{PA} are highly polymorphic. We cloned the third allele *vib-2*^{GR} which also shows a high polymorphism; *vib-2*^{GR} is more similar to *vib-2*^{PA} then *vib-2*^{OR}.

16. No sex in red light - A role for phytochromes in *Aspergillus nidulans*. Anne Blumenstein[1], Kay Vienken[1], Ronja Tasler[2], Nicole Frankenberg-Dinkel[2] and Reinhard Fischer[1], [1]University of Marburg and University of Karlsruhe, [2]University of Braunschweig.

Phytochromes are photoreceptors that sense red and far-red light through photo-interconversion between two stable conformations. This distinct feature is mediated by a covalently bound linear tetrapyrrole chromophore. Phytochromes were thought to be confined to photosynthetic organisms including cyanobacteria, but have been recently discovered in heterotrophic bacteria and in fungi, where little is known about their functions[1]. PhsA and orthologues from other fungi share chromophore-binding regions, which are phylogenetically closer related to bacteria than to plants, pointing to their evolution from a common bacterial ancestor. In support of that, all fungal phytochromes are multifunctional proteins, where the phytochrome region and histidine kinase domain are combined in a single protein with a C-terminal response regulator domain. We studied the role of the *Aspergillus nidulans* phytochrome (PhsA). PhsA expressed in *E. coli* autoassembled with biliverdin and less efficiently with phycocyanobilin. The functionality of the two-component system histidine kinase was shown in an in vitro kinase assay.

We found that *A. nidulans* PhsA acts as red-light sensor and represses sexual development under red-light conditions. PhsA-GFP was excluded from nuclei, suggesting that red-light perception occurs in the cytoplasm, in contrast to blue-light perception through the nuclear flavoprotein WC-1 in Neurospora crassa [2,3].

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17. Hyphal fusion in *Neurospora crassa*. Andre Fleissner, Sovan Sarkar and N. Louise Glass. University of California, Berkeley

The mycelial colony of filamentous fungi consists of a network of interconnected multinucleate hyphae. The colony grows by hyphal tip extension, branching and fusion (anastomosis). The ability to form hyphal fusions within one colony, but also between different individuals, enables fungi to establish complex functional units that show coordinated growth and exploration of their environment. To gain a better understanding of the basic, yet not well described, process of hyphal fusion, we are characterizing hyphal fusion mutants in *Neurospora crassa*.

The *soft* (*so*) mutant exhibits lack of anastomosis within a colony or between conidial germlings. We cloned the *so* gene by complementation. Database analysis showed that this gene is highly conserved in filamentous ascomycetes, but not present in ascomycete yeast species. The encoded protein contains a conserved WW-domain involved in protein-protein interactions. SO-GFP fusion proteins localize to the cytoplasm. Surprisingly the fusion protein localizes to the hyphal plugs in injured or dying cells. Overexpression of the *so* gene leads to a hyperbranching phenotype.

Using genetic analysis we are investigating the relationship of SO with a MAP-Kinase pathway involved in hyphal fusion. Furthermore we are analyzing the role and function of this signaling pathway by overexpression and introduction of activated alleles. GFP fusions are used to study the localization of these proteins during colony development.

18. Ramosa-1: An evolutionary conserved protein involved in fungal morphogenesis. S.J. Flitter[1], M. Arentshorst[1], C.G. Reynaga-Pena[3], S. Bartnicki-Garcia[4], C.A.M.J.J. van den Hondel[1,2], A.F.J. Ram[1,2]. [1]Leiden University, Institute of Biology Leiden, Fungal Genetics Research Group, Leiden, The Netherlands, [2]Department of Microbiology, TNO Nutrition and Food Research, Zeist, The Netherlands, [3]Dept. de Ing. Genetica Unidad Irapuato, Universidad de Guanajuato, Mexico, [4] Centro de Invesigacion Cientifica de Educacion Superior de Endenada, Mexico.

Polarised growth of leading hyphae of many filamentous fungi is characterised by the presence of a Spitzenkörper (Spk) or a Vesicle Supplying Centre (VSC). This structure is thought to allow the efficient delivery of vesicles to the growing apex. To identify proteins involved in this process we have characterised the previously isolated apical branching mutant, *ramosa-1* (1), and by complementation of the temperature sensitive phenotype have cloned the *ramosa-1* gene. Homology searches indicate that the protein belongs to an evolutionary conserved family of proteins, in all eukaryotic cells. The *ramosa-1* homolog gene in *S. cerevisiae*, YOL078w/AVO1, is an essential gene. Over-expression of the ramosa-1 cDNA using the GAL4 promoter could rescue the *S. cerevisiae* gene deletion mutant (deltaYol078w), indicating a functional conservation between the two homologs. Ramosa-1 depletion studies in *S. cerevisiae* indicate that the gene is required for proper morphogeneisis, as is seen in *Aspergillus niger*. Ramosa-1 homologs have been shown to be involved in several signalling mechanisms in the cell including MAPK signals (*S. pombe*), cyclic AMP signalling (*Dictyostelium*) and PI signaling (*S. cerevisiae*). Current research is aimed at the identification of Ramosa-1 interacting proteins to elucidate its function in fungal cells.

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19. Endosome based recycling of the pheromone receptor Pra1 is essential for pheromone perception in mating of the plant pathogen Ustilago maydis. Uta Fuchs and Gero Steinberg. Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse, 35043 Marburg, Germany

We previously identified the t-SNARE Yup1 as a key receptor for fusion of endocytic vesicles and an early endosomal compartment[1]. It was shown that endocytosis is involved in cellular morphogenesis but the relevance for this basic developmental process in pathogenic development of *Ustilago maydis* was not known. Here we demonstrate that Yup1-dependent endocytosis is essential for early infection stages of *U. maydis*. Temperature sensitive Yup1 mutants are non-pathogenic, and they are unable to form mating tubes in the presence of synthetic pheromone or the compatible mating partner. This is due to a defect in pheromone perception, while Yup1 only has a minor effect on growth and elongation of existing mating tubes. Pheromone perception in *Ustilago maydis* is based on the recognition of the pheromone receptor, Pra1/Pra2, and the compatible mfa2/mfa1 pheromone. In unstimulated cells low amounts of Pra1-GFP localize to the plasma membrane and are also found in the the vacuole suggesting that Pra1 is delivered to the vacuole for degradation. Upon pheromone stimulation Pra1 is enriched in the membrane of the growing tip of the mating tube. We show that Pra1 is endocytosed via actin-dependent processes and that it colocalizes with Yup1-tagged early endosomes. In temperature-sensitive Yup1 mutants Pra1 is still internalised but accumulates in the cytoplasm and is not transported to the vacuole. Here Pra1 is depleted from the plasma membrane, suggesting that the pheromone receptor is recycled back to the surface via Yup1-tagged early endosomes. These results argue for an important role of Yup1 mediated endosomal recycling of Pra1 in pheromone signalling and pathogenic development of *U. maydis*.

[1]Wedlich-Söldner, R., Bölker, M., Kahmann, R., Steinberg, G., (2000): A putative endosomal t-SNARE links exo- and endocytosis in the phytopathogenic fungus Ustilago maydis. EMBO J 19: 1974-1986.

20. Microtubules are dispensable for cell fusion but are essential for long distance hyphal growth in the corn smut fungus *Ustilago maydis*. Uta Fuchs, Isabel Manns and Gero Steinberg. Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse, 35043 Marburg, Germany

Fungal pathogenicity is often linked to a morphogenic switch between a yeast stage and a filamentous hypha. It is thought that the cytoskeleton underlies this transition, but a detailed understanding of the importance of microtubules and F-actin in morphological switch is missing. In *Ustilago maydis*, compatible yeast-like cells recognize each other in order to form conjugation hyphae that fuse and give rise to a b-dependent filament. Here we analyze the role of the cytoskeleton in these early steps of pathogenic development. We show that F-actin is essential for growth of yeast-like cells, cell fusion and formation of both conjugation and b-dependent hyphae. In contrast, microtubules have cell cycle-specific roles in both mitosis and cell polarity of yeast-like cells, but were of minor importance for hyphal growth. Conjugation hyphae elongate up to 100 m in length and contain long microtubules that extend their plus-ends towards the growing apex. However, in the absence of microtubules conjugation hyphae still elongate up to 60 m. A similar phenotype was observed in b-dependent hyphae, suggesting that microtubule based transport is dispensable for short distance hyphal growth. A quantitative analysis of fusion of compatible cells revealed that microtubules neither participate in pheromone recognition nor in cell-cell fusion, indicating that short conjugation hyphae are fully functional. These results demonstrate that F-actin has numerous crucial roles in dimorphic transition, while microtubules are essential for extended hyphal growth.

21. Analysis of dynein regulatory complexes using a tandem affinity purification tag for filamentous fungi. Kerstin Helmstaedt, Silke Busch, Ozgur Bayram, and Gerhard H. Braus. Institute of Microbiolgy and Genetics, Georg-August-University, Grisebachstr. 8, D-37077 Goettingen, Germany

In filamentous fungi, migration of nuclei is established through the interplay of the molecular motor dynein in combination with dynactin and microtubuli. Several proteins like NUDF, NUDE, and NUDC had been identified as regulators of dynein-mediated movement, which are not part of the dynein/dynactin motor complex itself (Xiang et al, 1995; Efimov and Morris, 2000). We intend to identifiy interaction partners of these regulatory proteins in *Aspergillus nidulans* in order to clarify the regulatory pathway leading to dynein activation. For this biochemical approach we used the tandem affinity purification (TAP) tag, which was originally developed for yeast and was optimized for expression in filamentous fungi. The nuclear migration gene *nudF* was fused to the new N-terminal tag and expressed in *A. nidulans* from its native promoter for gentle purification of active regulatory complexes at their natural level. Proteins which co-purified with NUDF were identified by mass spectrometry. In first affinity purifications, several hypothetical proteins with homologs in other filamentous fungi and higher eukaryotes, respectively, were found. The method is currently improved and interactions are verified by yeast two-hybrid analysis.

22. White collar homologs sense blue light in basidiomycetes and zygomycetes. Alexander Idnurm and Joseph Heitman. Department of Molecular Genetics and Microbiology, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710, USA.

Light is the primary energy source for life on earth and as such is a major environmental signal for organisms from all Kingdoms of life, including the fungi. Through candidate gene and insertional mutagenesis approaches we have elucidated the function of two genes whose products perceive light and control mating mating in the basidiomycete human pathogen *Cryptococcus neoformans*. Light represses mating of *C. neoformans*, and strains mutated in a homolog of *Neurospora crassa white collar 1* (*BWC1*) mated equally well in the light or the dark. The predicted Bwc1 protein shares identity with *N. crassa* WC-1, but lacks the zinc finger DNA binding domain. In *C. neoformans* Bwc1 regulates cell fusion and represses hyphal development after fusion in response to blue light. In addition, *bwc1* mutant strains are hypersensitive to ultraviolet light. To identify other light sensing components, a novel self-fertile haploid strain was created and subjected to *Agrobacterium*-mediated insertional mutagenesis. One UV-sensitive mutant that filaments equally well in the light and the dark was identified and found to have an insertion in the *BWC2* gene, whose product is structurally similar to *N. crassa* WC-2. Finally, we analyzed the zygomycete *Phycomyces blakesleeanus*, and found two homologs of WC-1. Sequence analysis is underway to determine whether or not these homologs are mutated in the photon insensitive *mad* strains. These results demonstrate that perception of UV/blue light via the White collar system is an ancient process that predates the divergence of the fungi into the ascomycete, basidiomycete and zygomycete phyla.

23. The cAMP signaling pathway is involved in osmotic tolerance, but not in dicarboximide resistance in *Cochliobolus heterostrophus*. Kousuke Izumitsu, Akira Yoshimi, and Chihiro Tanaka. Laboratory of Environmental Mycoscience, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan.

In the fungus *Cochliobolus heterostrophus* (anamorph: *Bipolaris maydis*), the mutants resistant to dicarboximide fungicides are sensitive to high osmolarity (Yoshimi et al. 2003). Genetic analysis indicated that three genes were associated with osmosensitivity and dicarboximide resistance: *Dic1*, *Dic2*, *Dic3*. *Dic1* encoded the histidine kinase that confered osmotic adaptation and dicarboximide resistance, but the functions of *Dic2* and *Dic3* are unknown. In *Ustilago maydis*, it was reported that mutants with disruptions in the *ubc1* gene, which encoded the regulatory subunit of PKA (cAMP dependent protein kinase), were resistant to dicarboximide and sensitive to high osmolarity (Marilee et al. 2001). To evaluate the role of cAMP signaling pathway in *C. heterostrophus*, we isolated and characterized two genes involved in cAMP signaling pathway. These two genes, *BmRpk1* and *BmCyr1*, encoded a PKA regulatory subunit and adenylyl cyclase, respectively. Disruption of *BmCyr1* resulted in the significant reduction of conidiation, whereas *bmrpk1* mutants showed normal conidiation. Both *bmcyr1* mutants and *bmrpk1* mutants show sensitivity to dicarboximide, similar to the wild-type strain. This result suggests that, unlike in the case of *U. maydis*, the cAMP signaling pathway do not mediate resistance to dicarboximide in *C. heterostrophus*. With regard to the sensitivity to osmotic stress, the growth of the *bmrpk1* mutants was significantly reduced on the medium amended with 0.8M KCl, comparing with that of the wild-type strain. This suggests that the cAMP signaling pathway is involved in osmotic adaptation in *C. heterostrophus*.

24. The absence of meiotic silencing by unpaired DNA (MSUD) in *Neurospora tetrasperma*. David Jacobson and Namboori Raju. Department of Biological Sciences, Stanford University, California.

Genes that are unpaired during meiosis are silenced in *N. crassa*. GFP-tagged *histone H1* when inserted at the *his-3* locus on linkage group I (LGI) allows visualization of MSUD in developing asci by fluorescence microscopy. When homozygous, *hH1::GFP* is paired during meiosis; it expresses normally and nuclei fluoresce throughout ascus development. However, when heterozygous, it is unpaired and silenced during meiosis until ascospore demilitation. MSUD does not extend into the ascospore maturation stage and *hH1::GFP* nuclei fluoresce in four of the eight ascospores. *N. tetrasperma* packages two nuclei of opposite mating type into each of its four ascospores. This is accomplished by blocking recombination in a large region of LGI. LGI also shows a large unpaired region during pachytene. To test whether the genes in this unpaired region are silenced, *hH1::GFP* at *his-3* was introgressed from *N. crassa* into *N. tetrasperma*. The initial hybrid cross produced almost all 8-spored asci with a high level of ascospore abortion, but showed no silencing of *hH1::GFP*. After four backcrosses to *N.tetrasperma*, all progeny were phenotypically *N. tetrasperma*: asci were 4-spored and ascospores were heterokarotic for *hH1::GFP*. All nuclei in the developing asci and in the heterokaryotic ascospores fluoresced brightly. Thus, unpaired *hH1::GFP* on LGI is not silenced in *N. tetrasperma*. The absence of MSUD in *N. tetrasperma* is genome wide, as *hH1::GFP* constructs inserted on other chromosomes (courtesy of M. Freitag, U. Oregon) were also not silenced. *Sad-1* is a suppressor of MSUD in *N. crassa*, and its role in *N. tetrasperma* is under investigation.

25. Molecular analysis of *snoA* (suppressor-of-nimO), a novel regulator of DNA synthesis in *Aspergillus nidulans*. Elizabeth Wille, Matthew Denholtz, Allison Altenburger, and Steve James. Department of Biology, Gettysburg College, Gettysburg, PA 17325

In *Aspergillus nidulans*, *nimO*^{Dbf4} and *cdc*7encode regulatory and catalytic subunits of the conserved Dbf4-dependent kinase (DDK). DDK initiaties DNA synthesis by phosphorylating components of DNA helicase to trigger origin unwinding. The temperature sensitive *nimO*18 allele can be partially suppressed by mutations in two genes, *snoA* and *snoB*. Semi-dominant *snoB* mutations lie within the *cdc*7 gene, and suppress by stabilizing the association of cdc7p with nimO18p. Recessive *snoA* suppressors not only rescue *nimO*18 ts-lethality, but they also rescue growth of strains hypomorphic for expression of *nimO*$^{+}$. However, *snoA* suppressors cannot bypass a complete loss of *nimO*. Thus, *snoA* suppression is not allele-specific, and *snoA* appears to act by influencing the level or stability of nimOp. Bioinformatic and positional cloning approaches were combined to localize *snoA* within a 340,000 bp interval on Linkage Group VII. *snoA* was discovered by systematic ORF deletion, in which deletion of one ORF phenocopied the *snoA* suppressor phenotype by partially rescuing *nimO*18 ts-lethality. Current efforts include *snoA* overexpression studies, mapping *snoA* functional domains, and biochemical analysis of nimOp and cdc7p in *snoA* mutants. (Supported by NSF-RUI #01-14446)

26. Endocytosis: A Filamentous Fungal Perspective. E.R. Kalkman[1], Robert W. Robertson[2], and N.D. Read[1] .[1]Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, Rutherford Building, Edinburgh EH9 3JH, UK. [2]Department of Plant Biology, Arizona State University, Tempe, Arizona, USA

Endocytosis has been well characterized in budding yeast and animal cells, and to a lesser extent in plant cells. In contrast, much less is known about this process in filamentous fungi although it is likely to have important roles in membrane recycling, membrane degradation, and the uptake of signal molecules. This presentation will provide evidence from studies of several different filamentous fungi, most notably Neurospora crassa, which support the occurrence of endocytosis in filamentous fungi. This evidence is: 1) Membrane-selective markers of endocytosis (FM4-64) are internalized; 2) A marker of fluid-phase endocytosis (Lucifer Yellow) is internalized; 3) Internalization of endocytosis markers is an active process and not diffusion since it is reversibly inhibited by azide or cold treatment; 4) Internalization of FM4-64 is actin-mediated because it is inhibited by Latrunculin B; 5) The genomes of several filamentous fungi encode complex endocytic protein machineries

27. Comparison of membranes imaged with fluorescent probes FM4-64 and DiOC6 to GFP-tagged ER, Golgi and microtubules in *Aspergillus nidulans*. Michelle Hubbard and Susan Kaminskyj, Dept. Biology, Univ Saskatchewan, Saskatoon, SK S7N 5E2, Canada

Fungal tip growth depends on precisely targeted secretion of endomembrane-derived vesicles. The *Aspergillus nidulans hypA* and *hypB* morphogenesis loci have roles in ER-Golgi transport via the COPII and COPI pathways, respectively. To facilitate future work in mutant phenotype studies, we imaged *A. nidulans* endomembranes with the lipophilic dyes FM4-64 and DiOC6 using confocal microscopy. Arrays were compared with GFP-tagged ER, Golgi and microtubule patterns. Effective DiOC6 concentrations were 1000x lower than FM4-64. FM4-64 patterns changed over time, first staining the cell membrane and later being internalized; DiO6 staining of endomembranes was rapid. In growing wildtype cells there was overall agreement between FM4-64 and DiOC6 patterns after 1 h incubation in FM4-64, except that DiOC6 did not label a putative apical vesicle cluster. Time lapse images with both dyes showed endomembrane movements. Although theoretically possible, we were not able to effectively unmix signals from DiOC6 and GFP-tagged cytoplasmic microtubule arrays due to spatial rather than spectral constraints. FM4-64 arrays co-localized well with GFP-ER, but generally not with GFP-Golgi. Comparing endomembrane and cytoplasmic microtubule arrays in developing branches in wildtype and *hypA1* mutants, we find that Golgi cluster at branch initiation sites, whereas both Golgi and cytoplasmic microtubules have roles in branch extension.

28. A *Sec7* domain gene has a role in *Aspergillus nidulans* morphogenesis. Lifeng Chen, Yi Yang, Susan Kaminskyj. Dept Biology, Univ Saskatchewan, Saskatoon, SK S7N 5E2, Canada

A *Sec7* domain containing sequence was isolated as an extragenic suppressor of the *Aspergillus nidulans hypB5* temperature sensitive morphogenesis defect. A *hypB5* mutant strain was transformed with the pRG3-AMA1 library. A complementing plasmid was rescued, and a 5 kb *Kpn*I fragment containing the complementing region was cloned into pBluescript as pYY2. The restrictive phenotype of *hypB5* mutant strains is restricted colonial growth, and slow-growing cells with aberrant branching. The pYY2-transformed strains have wildtype colonies at 42C. At 42C these hyphae have shorter basal cells with more nuclei than the wildtype parent strain A28. An intact *Sec7*domain is required for wildtype colony growth: pYY2 with Tn1000-disruptions in, but not adjacent to, the *Sec7* domain failed to rescue the *hypB5* temperature sensitive defect. The pYY2 *Sec7* domain is 81% identical to *Saccharomyces cerevisiae SEC7*. In *A. nidulans* it is located in the predicted protein An6709.2. Structural modeling shows good theoretical agreement with the *Sec7* domain of human ARNO (ADP Ribosylation Nucleotide Opener). The *Sec7* domain is 10% of the predicted length of An6709.2; the remainder of the predicted gene lacks homologies to characterized sequences. In other *Sec7* genes, the conserved F-G loop is critical for function. We are using site-directed mutagenesis to examine effects of single residue replacements on hyphal morphogenesis.

29. Proteins binding to subterminal repeats of transposon *Restless*. Ilka Braumann and Frank Kempken. Abteilung für botanische Genetik und Molekularbiologie, Botanisches Institut und Botanischer Garten, Christian-Albrechts-Universität Kiel, Am Botanischen Garten 1-9, 24118 Kiel, Germany

The fungal transposon *Restless* is a member of the *hAT* family of eukaryotic transposable elements (1,2). In previous studies we have demonstrated the activity of *Restless* and its use for gene tagging in its host *Tolypocladium inflatum* (3). In addition we observed alternative splicing (4), excision, and the generation of deleted transposon copies (5) in the foreign host *Neurospora crassa*.
To further analyse the function and transposition of *Restless* we set out to identify cis-acting elements involved in transposition. ^{32}P-labeled PCR fragments of *Restless* terminal and subterminal regions were used for gel shift analysis. UV cross linking experiments were employed to identify *Restless*-binding proteins. Results of these experiments will be shown and discussed. In addition we present data regarding the expression of the *Restless* transposase.
(1) Kempken F, Windhofer F (2001) Chromosoma 110:1-9, (2) Kempken F (2003) In: Arora DK, Khachatourians GG (eds) Applied Mycology and Biotechnology, Vol. 3 Fungal Genomics, Elsevier Science Annual Review Series, pp83-99, (3) Kempken F, Kück U (2000) Mol Gen Genet 263:302-308, (4) Kempken F, Windhofer F (2004) Current Genetics 46:59-65, (5) Windhofer F, Hauck K, Catcheside DEA, Kück U, Kempken F (2002) Fungal Genet Biol 35:171-182

30 Ambient pH signaling influences phosphate acquisition in *Neurospora crassa*. Patrick W. Kennedy[1], Robert L. Metzenberg[2], Maria J. Harrison[3] and Wayne K. Versaw[1]. [1]Dept of Biology, Texas A&M University, College Station, TX [2]Dept of Chemistry and Biochemistry, University of California, Los Angeles, CA [3]Boyce Thompson Institute for Plant Research, Cornell University, Ithica,

N. crassa exhibits two modes of phosphate uptake – constitutive, low-affinity transport and phosphate-repressible, high-affinity transport. The two transport systems can be operationally distinguished not only by available phosphate concentration but also by ambient pH. At pH values above neutrality, the Km of the low-affinity system rises sharply so that this system is unable to support growth under modest phosphate concentrations without the assistance of at least one component of the high-affinity system. Thus a mutant lacking the high-affinity transporters PHO-4 and PHO-5 is unable to grow under the restrictive conditions. The identity of the transporter(s) that comprise the low-affinity system has not been reported but a mutant isolated as a suppressor of the *pho-4; pho-5* double mutant's growth defect displays altered phosphate transport activity. Complementation of this mutant revealed that the affected gene encodes a protein homologous to PalF of *Aspergillus nidulans*, which is one component of a highly conserved, ambient pH signaling pathway. Deletion of the *pacC* homolog, another component of the same pathway, gives rise to an identical phenotype indicating that pH signaling has a role in modulating low-affinity phosphate transport.

31. Pheromone receptor genes are essential for mating type-specific trichogyne chemotropism and female fertility, and are also involved in perithecial development in *Neurospora crassa*. Hyojeong Kim and Katherine A. Borkovich. UC Riverside.
In the heterothallic ascomycete *Neurospora crassa*, mating involves differentiation of protoperithecia and chemotropic growth of trichogynes towards opposite mating type cells in a pheromone-mediated process. The pheromone receptor genes, *pre-1* and *pre-2*, are predicted to encode G-protein-coupled receptors with sequence similarity to other fungal pheromone receptors. Northern analysis indicates that *pre-1* and *pre-2* are preferentially expressed in *mat A* and *mat a* strains, respectively, under mating conditions. Deletion of *pre-1*, *pre-2* or both does not greatly affect vegetative growth, heterokaryon formation or male fertility in either mating type. During sexual development, delta *pre-1*, delta *pre-2* and delta *pre-1* delta *pre-2* strains form normal protoperithecia and are fertile as males. However, protoperithecia from *pre-1 mat A*, *pre-2 mat a* and both mating types of *pre-1 pre-2* double mutants do not undergo fertilization, due to inability of their trichogynes to recognize and fuse with opposite mating-type cells. Consistent with a requirement for GNA-1 (G alpha subunit) and GNB-1 (G beta protein) for female fertility in *N. crassa*, trichogynes of *gna-1* and *gnb-1* mutants display defects in growth towards and fusion with male cells. Results from studies involving forced heterokaryons of double mutants or inappropriate expression of the receptors suggest that one compatible pheromone/pheromone receptor combination is sufficient for perithecial development in *N. crassa*.

32 GPR-1, a G-protein coupled receptor, regulates sexual development in *Neurospora crassa*. Svetlana Krystofova and Katherine A. Borkovich, Dept. Plant Pathology, University of California Riverside, Riverside

The analysis of the genome sequence revealed that *Neurospora crassa* has at least 10 G-protein coupled receptors (GPCRs), including a novel gene family consisting of the GPCRs GPR-1, GPR-2 and GPR-3. Predicted protein sequences of these receptors share similarity to GCR1 from *Arabidopsis* and CrlA, CrlB and CrlC from *Dictyostelium*. The *gpr-1*, *gpr-2* and *gpr-3* genes are expressed at various developmental stages of the *N. crassa* life cycle. The highest expression of *gpr-1* was detected in sexual tissues. Deletion of *gpr-1* leads to pleiotropic defects during sexual development. Protoperithecia are pale and significant number are small and buried in solid medium. Perithecia have deformed beaks and often lack ostioles. Studies are in progress to determine possible downstream effectors of GPR-1, as well as genes that might be regulated by GPR-1.

33. The *Colletotrichum lagenarium* Genes ClaKEL1 and ClaKEL2 are Involved in Appressorium Morphogenesis. Yasuyuki Kubo, Kyoto Prefectural University, Laboratory of Plant Pathology, Shimogamo Japan

Colletotrichum lagenarium is the causal agent of cucumber anthracnose disease. The infection process involves a series of change in fungal morphology. We have isolated two novel genes, ClaKEL1 and ClaKEL2, from C. lagenarium. ClaKEL1 was disrupted in the pathogenicity mutant P24 generated by restriction enzyme-mediated integration. ClaKEL1 encodes a putative protein of 555 amino acids containing kelch motif at the C teminal region. To define the function of ClaKEL1, we isolated clakel1 knock-out mutants by homologous recombination. The clakel1 disruptant produced less conidia than the wild type on PDA medium, conidia formed malformed appressoria on glass slides, and showed weak pathogenicity on cucumber leaves. A second gene containing the kelch motif, ClaKEL2, was isolated by PCR using degenerate oligonucleotide primers based on the kelch motif. ClaKEL2 encoded a putative protein of 1577 amino acids containing the kelch motif at the N terminal region, and had significant homology with Saccharomyces cerevisiae KEL1 which has a role in cell morphogenesis and cell fusion, and Schizosaccharomyces pombe TEA1, which is required for microtubule dynamics and proper rod like cell morphology. The phenotype of clakel2 knock-out mutants was impaired maintenance of conidial dormancy. On glass slides, conidia formed normal melanized appressoria but most of them underwent lateral germination, and failed to penetrate cellulose membranes. These results indicate that ClaKEL1 and ClaKEL2 are involved in appressorium morphogenesis.

34. Cytoplasmic dynein is targeted to the spindle poles during mitosis. Shihe Li*, C. Elizabeth Oakley#, Guifang Chen*, Xiaoyan Han*##, Berl R. Oakley# and Xin Xiang*. *Department of Biochemistry and Molecular Biology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814. #Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210. ## Richard Montgomery High School, Rockville, MD 20852.

In *Aspergillus nidulans,* the spindle-pole localization of cytoplasmic dynein and its regulator NUDF/LIS1 is regulated in an interesting manner during mitosis. While NUDF localizes to the poles of spindles of various lengths, cytoplasmic dynein's spindle pole localization is more apparent on longer spindles, and is dependent upon the function of BIME/APC1, a component of the anaphase-promoting complex (APC). Moreover, while NUDF's localization to the spindle poles is not affected by a loss-of-function mutation of the cytoplasmic dynein heavy chain, the spindle-pole localization of cytoplasmic dynein is greatly diminished in a nudF loss-of-function mutant, suggesting that the function of NUDF is required for cytoplasmic dynein's targeting to the spindle poles. The localization of either NUDF or cytoplasmic dynein is not sensitive to transient microtubule depolymerization, suggesting that cytoplasmic dynein and NUDF bind directly to the spindle pole body (SPB) during mitosis. Interestingly, a gamma-tubulin mutation, mipAR63A, significantly eliminates the localization of cytoplasmic dynein to the spindle poles, while it has no apparent effect on NUDF's spindle-pole localization. These results suggest that the targeting of dynein, and possibly its cargoes, to the SPB is regulated during mitosis.

35. Characterization of *Aspergillus nidulans* mutants that display altered responses to a novel bacterial anti-fungal compound. Shaojie Li, Gary Y. Yuen, and Steven D. Harris, University of Nebraska, Lincoln, NE 68583

HSAF is a novel antifungal lipopeptide produced by a bacterial biocontrol agent, Lysobacter enzymogenes strain C3. HSAF can confer protection against plant pathogens though its mode of action remains unknown. Here, we show that HSAF induces dramatic morphological changes in germinating Aspergillus nidulans spores and growing hyphae. These include formation of multiple branches, aberrant accumulation of cell wall material and abnormal patterns of action organization. Genetic analysis shows that dominant mutations affecting at least two genes, barA and barB, cause resistance to HSAF, and a recessive mutation affecting the basA gene cause hypersensitivity to HSAF. The barA mutant also displays recessive temperature sensitive growth and morphological defects consistent with failure to maintain hyphal polarity. Molecular characterization of barA revealed that it is the A. nidulans homologue of yeast Lag1, which is essential for Acyl-CoA-dependent ceramide synthesis. Supplementation experiments strongly suggest that BarA is functionally analogous to Lag1p. The basA mutant fails to maintain hyphal polarity and displays aberrant accumulation of cell wall material at restrictive temperature. BasA is the A. nidulans homologue of yeast Sur2, sphinganine C4-hydroxylase, which catalyses the conversion of sphinganine to phytosphingosine. Supplementation experiments strongly suggest that BasA is functionally analogous to Sur2p. Collectively, these results suggest that the mode of HSAF action is related to sphingolipid synthesis.

36. A putative carbon sensing G protein coupled receptor. Liande Li and Katherine A Borkovich. Plant Pathology, University of California Riverside

At least 10 seven-transmembrane helix GPCRs have been predicted in the *N. crassa* genome (Borkovich et al, 2004). One of the GPCRs is named GPR-4 which is most similar to the putative sugar sensory receptors in yeasts. The *gpr-4* gene structure was verified by RT-PCR, which contains two exons and one intron. Expression analysis indicated that *gpr-4* expression level is very low. Quantitative RT-PCR showed that *gpr-4* has the highest level of expression in 3 day solid cultures. *gpr-4* mutants have different response to different carbon sources, suggesting that GPR-4 may act as a carbon source sensor. The complemented *gpr-4* strain could totally or partially rescue the phenotype of delta *gpr-4* to wild type level. Epistasis analyses showed that GPR-4 is upstream of GNA-1, not GNA-2 nor GNA-3, which indicate that GPR-4 is probably coupled to GNA-1, but not GNA-3 nor GNA-2. *gpr-4* mutants also share characteristics with *gna-1* mutants on such aspects as premature conidiation, H_2O_2 resistance and induced thermotolerance in germlings, and so on. Exogenous cAMP could partially rescue the phenotypes of delta *gpr-4* . Taken together, this research showed that GPR-4 may act as a carbon sensor, may couple to GNA-1 and is likely to be in a carbon-sensing GPCR, Galpha protein-adenylyl cyclase-cAMP-PKA pathway. More experiments are in progress to further prove this hypothesis.

37. A single septin gene from *Aspergillus nidulans* induces filamentous growth in *Saccharomyces cerevisiae*. Rebecca Lindsey, Youngsil Ha and Michelle Momany* Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA

Septins were first discovered in *S. cerevisiae* where they are thought to form a scaffold that organizes the bud site and they are a component of the morphogenesis checkpoint that monitors bud shape and coordinates budding with cytokinesis. Here we show that one of the A. nidulans septins, AspC, induces filaments and elongated asci containing up to twelve spores in S. cerevisiae. AspC-induced filaments are noninvasive, form in haploid and diploid strains and require the presence of a functional yeast septin Cdc10. Based on comparisons of amino acid sequence, AspC is most similar to the S. cerevisiae septin Cdc12. The A. nidulans aspC gene complements cdc12 and cdc3 yeast mutants. When GFP-Cdc12 and AspC are co-expressed Cdc12 localizes to the necks of buds, but it does not localize to the necks of AspC-induced filaments. In budding yeast, the Bni1 formin and Cdc12 are known to interact. When aspC is introduced into an S. cerevisiae bni1 delete strain, a novel bent filament phenotype is seen. This phenotype is suppressed by extra copies of CDC12. Our results suggest that the A. nidulans septin AspC competes with Cdc12 for incorporation into the septin scaffold at the S. cerevisiae neck and that when AspC is present it perturbs normal yeast bud morphology causing it to take on the more elongated shape common to filamentous fungi.

38. Novel regulators of septation in *Aspergillus nidulans*. Ling Lu, Rongzhong Shao, Jung-Mi Kim, and Bo Liu. Section of Plant Biology, University of California, Davis, CA 95616, USA

The kinase cascade of the septation initiation network (SIN)/ mitotic exit network (MEN) plays a regulatory role for septation in fungi. The evolutionarily conserved MOB1 protein is a novel protein that is associated with the terminal kinase of this cascade. The exact role of MOB1 is unclear. In the filamentous fungus *A. nidulans*, AnMOB1 is required for septation and conidiation, but is not essential for hyphal extension and colony formation. Because the *A. nidulans* mycelium contains multinucleate cells, novel mechanisms may exist to regulate septation in this organism. To identify novel septation regulators in *A. nidulans*, by UV mutagenesis we have isolated suppressor (*smo*) mutations that restored conidiation when *AnMOB1* was down-regulated/not expressed. Microscopic examination indicated that the restored conidiation was concomitant with restored septation in the absence of the AnMOB1 protein. Among 110 independent *smo* mutants, five in two complementation groups demonstrated reduced hyphal growth, colony formation, and conidiation in the presence of AnMOB1. These five *smo* mutations also rendered hypersensitivity to low doses of the microtubule-depolymerizing agent benomyl. However, none of these *smo* mutations altered the localization of AnMOB1. Therefore, regulators antagonizing the SIN/MEN pathway likely exist in *A. nidulans*. Isolation of *smo* genes will shed light on regulatory mechanisms underlying septation in filamentous fungi. Progresses on characterization of the *smo* mutants, and identification of the *smo* genes will be presented. This work was supported by the NSF.

39. The *csnD-E* signalosome genes are involved in the *Aspergillus nidulans* DNA damage response. Iran Malavazi[1], Joel F. Lima[1], Marcia R.V.Z.K. Fagundes[1], Marcela Savoldi[1], Maria H. S. Goldman[2], Gerhard Braus[3] and Gustavo H. Goldman[1]. [1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto and [2]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Brazil. [3]Department of Microbiology and Genetics, Institute for Microbiology and Genetics, Georg-August-University Gottingen, Germany.

The signalosome (CSN) is a conserved complex involved in protein turnover and eukaryotic development, and is also required to activate ribonucleotide reductase for DNA synthesis. In *A. nidulans*, *csnD* and *csnE*, are key regulators of sexual development. We investigated if these genes are involved in the DNA damage response. The growth of the *csnD-E* deletion mutants is reduced by sub-inhibitory concentrations of camptothecin (CPT) and 4-nitroquinoline oxide (4-NQO). In *A. nidulans*, septum formation is inhibited by DNA damage in a checkpoint-dependent manner. Nevertheless, septation is not inhibited in the *csnD-E* deletion mutants upon DNA damage caused by methyl methane sulfonate (MMS). The *csnD* mRNA expression was induced by CPT, MMS, bleomycin (BLEO) (about 3-fold), and 4-NQO (9-fold). The *csnE* gene encoding the deneddylase activity which is required to modify E3 SCF ubiquitin ligases, has high levels of mRNA expression induced by all drugs (from 10-fold, CPT to 37-fold, 4-NQO). Germinating conidia of *csnD-E* deletion strains are more sensitive to ultraviolet light than the corresponding wild type strain. We constructed double mutants with *csnD-E* deletion, *npkA* and *uvsB*^{ATR}. The NpkA is a cdc2-related kinase that is involved in the S-phase. The double *csnD npkA* and *csnE npkA* mutants are much more sensitive to DNA damaging agents than the respective single mutants. However, the intra-S-phase and DNA replication checkpoints are intact in these mutants. Our results suggest that *csnD-E* genes are involved in the DNA damage response and that NpkA genetically interacts with the CSN.
Financial support: FAPESP and CNPq, Brazil

40. Studies of the *Neurospora crassa* adenylyl cyclase, CR-1. Sara Martinez and Katherine Borkovich. University of California, Riverside.

Heterotrimeric G proteins are involved in the ability of eukaryotic cells to react to various stimuli. In pathogenic filamentous fungi, G proteins are essential for virulence. In the non-pathogenic filamentous fungus *Neurospora crassa*, there are three G alpha subunits (GNA-1, GNA-2, GNA-3), one G beta subunit (GNB-1) and one G gamma subunit (GNG-1). One of the proteins acting downstream of G proteins is the adenylyl cyclase, CR-1. The G alpha subunits have been shown to regulate CR-1 level and function. CR-1 activity in *gna-1* deletion mutants is decreased compared to wild type, but protein levels are equal, suggesting GNA-1 affects CR-1 function. In *gna-3* deletion mutants, both activity and level of CR-1 is significantly lower than wild type, indicating that a loss of GNA-3 causes a decrease in CR-1 protein levels. GNA-2 is thought to function as a compensatory protein to GNA-1 and GNA-3, and levels and activity of adenylyl cyclase are normal in *gna-2* deletion mutants. Furthermore, the *cr-1* mutant has more severe forms of phenotypes observed in *gna-1* mutants. This data suggests that GNA-1 could be directly interacting with CR-1. Results will be presented from experiments testing for interactions between the CR-1 and G proteins. The understanding of how CR-1 functions, its role in G protein signaling, and its downstream effectors will give insight as to how eukaryotes respond to stimuli. This data can be applied to pathogenic fungi as well as mammalian studies to aid in the fight of plant and animal disease.

41. **The *snxA1* and *nimA5* mutations of *Aspergillus nidulans* Interact to Affect Mitotic Spindle Structure.** R. Day, B. Fontenelle, S. Chandna, and S.L. McGuire, Millsaps College, Jackson, MS, USA

The *snxA* gene interacts with *NIMX*^{cdc2} to affect mitosis, and its mutation causes abnormal nuclear morphology at 17C, while the *nimA* gene affects the nuclear import of NIMX^{cdc2} and when mutated blocks mitotic entry at 42C. To characterize the effects of the *snxA1* mutation on microtubule and nuclear structure, strains expressing a GFP-tubA (alpha-tubulin) gene were generated with either *snxA1* or *nimA5* mutations alone or with a *snxA1/nimA5* double mutant. At 17C *snxA1*/GFP-tubA cells had severe nuclear defects, thickened hyphae, abnormal spindle structures, and abnormal interphase microtubule arrays. Mitotic spindles were highly variable in length. Some spindles had no nuclei attached to them, while yet others were bifurcated or trifurcated and had fragmented, variably condensed nuclei along their lengths. Similar abnormal nuclei and spindle structures were observed when *snxA1/nimA5* strains were germinated at 32C and upshifted to 42C for 3 hours. Additionally, *snxA1* was shown to suppress a checkpoint defect observed in *nimA5* cells: Double mutants were able to grow in the presence of 20mM hydroxyurea, while *nimA5* cells were sensitive at 10mM hydroxyurea. Cloning and further characterization of the *snxA* gene will provide clues to the interactions of NIMXCDC2, NIMA, SNXA, and the regulation of mitotic spindle formation. Supported by NIHGM55885-03 and the Mississippi Functional Genomics Network.

42. The isolation and identification of a Cephalosporin transporter homologue in *Penicillium chrysogenum*. Nijland JG, Evers ME, Driessen AJM. Department of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands.

The pathway of the biosynthesis of penicillin and the cellular localisation of the critical enzymatic steps has been elucidated. Little is known about the transport processes that play a role in beta-lactam excretion by Penicillium chrysogenum. Overexpression of the CefT gene in Acremonium chrysogenum resulted in a two-fold increase in cephalosporin C production in this organism. Therefore, we have used a PCR-based cloning strategy to identify transporters involved in antibiotics transport in P. chrysogenum. By means of sets of degenerate primers, a MFS transporter homologue was identified that is expressed under conditions of beta-lactam production. The full length CefT gene was obtained from a phage DNA library and showed homology (53%) to the CefT in A. chrysogenum. Expression studies revealed the increased level of mRNA under penicillin inducing conditions of the CefT homologue.
Expression of the CefT gene of A. chrysogenum and the homologues gene of P. chrysogenum, both fused to GFP were overexpressed in P. chrysogenum. Both genes show localisation at the plasma membrane.
To determine the substrate specificity of the CefT of A. chrysogenum it was also expressed in E.coli and in insect cells. By the use radiolabeled derivatives of cephalosporin, the transport activity will be determined.

43. Disordered cell integrity signaling caused by disruption of the *kexB* gene in *Aspergillus oryzae*. Osamu Mizutani, Tomonori Fujioka, Youhei Yamagata, Keietsu Abe, and Tasuku Nakajima. Molecular and Cell Biology, Tohoku University, Sendai, Japan

To examine the physiological role of *kexB*, which encodes a subtilisin-like processing enzyme, in *A. oryzae*, we constructed a *kexB* disruptant (delta-*kexB*), which formed shrunken colonies with poor conidia generation on Czapek-Dox (CD) agar plates. The phenotypes of the delta-*kexB* strain were restored under high-osmolarity in solid culture conditions. We found that transcription of the *mpkA*, which encodes a putative mitogen-activated protein kinase involved in cell integrity signaling, was significantly higher in delta-*kexB* cells than in wild-type cells. The delta-*kexB* cells also contained higher levels of transcripts for cell wall-related genes encoding *ss*-1,3-glucanosyltransferase and chitin synthases. As expected, constitutively increased levels of phosphorylated MpkA were observed in delta-*kexB* cells on the CD plate culture. High osmotic stress greatly downregulated the increased levels of both transcripts of *mpkA* and phosphorylated form of MpkA in delta-*kexB* cells, concomitantly suppressing the morphological defects. These results suggest that the upregulation of transcription levels of *mpkA* and cell wall biogenesis genes in the delta-*kexB* strain is autoregulated by phosphorylated MpkA as the active form through cell integrity signaling. We think that KexB is required for precise proteolytic processing of sensor proteins in the cell integrity pathway or of cell wall-related enzymes under transcriptional control by the pathway and that the KexB defect thus induces disordered cell integrity signaling.

44. Vegetative incompatiblity, TOR pathway and autophagy in *Podospora anserina*. Bérangère Pinan-Lucarré and Corinne Clavé, IBGC UMR 5095 CNRS, laboratoire de génétique moléculaire des champignons, 33077 Bordeaux France.

Vegetative incompatibility is an ubiquitous phenomenon in filamentous fungi. It prevents the formation of viable heterokaryons between strains non-isogenic at *het* loci. The heterokaryon resulting from cell fusion is rapidly destroyed by a cell death reaction. A main feature of the incompatibility reaction is the intense vacuolization of the cytoplasm. Autophagy is a pathway allowing degradation of macromolecules and organelles in vacuoles. It is also associated with type II programmed cell death. We have shown that autophagy is induced during incompatibility. We propose to determine if autophagy is responsible for cell death by incompatibility. Therefore we constructed a mutant strain disrupted for the autophagy gene *atg1-Pa*. We have got cytological evidence that autophagy is blocked in this mutant. Surprisingly, this mutant is not impaired in rapid and intense vacuolization of the cytoplasm and the incompatibility reaction still occurs. In eukaryotes, autophagy is negatively regulated by TOR kinase, a central growth controller. Treatment of Podospora with rapamycin, a highly specific TOR inhibitor in most eukaryotes, induces a lot of features of incompatibility including cell death. We proposed that interaction between incompatible *het* genes results in *Pa-tor* inactivation.
In order to ensure that rapamycin has also a direct effect on TOR in Podospora, we constructed a mutant allele by site directed mutagenesis in the conserved rapamycin binding domain. Transformation of Podospora with this allele confers rapamycin resistance. This indicates that the toxicity of rapamycin is mediated via the TOR pathway in Podospora.

45. Ceramide Signals Death in the Heat-Stress Response. Nora Plesofsky and Robert Brambl, Department of Plant Biology, University of Minnesota, St. Paul, MN 55108.

Neurospora crassa displays a typical heat shock response at 45ºC, synthesizing the major heat shock proteins strongly, but transiently. This response allows *Neurospora* to survive an otherwise lethal exposure (2 hr) to 50ºC. More recently we have found conditions that are lethal for *Neurospora* when it is exposed to 44.5ºC, by adding inhibitory glucose analogs, thereby reducing availability of carbohydrate and energy stores. Surprisingly, the lethality of this combined heat and carbon stress depends on biotin being present in the culture medium; without biotin the cells survive the combined stresses. The response of relevant mutant strains of *Neurospora* indicates that fatty acid synthesis is required for lethality. To identify the type of lipid molecule responsible for the stress-caused death, we added various fatty acids and sphingolipids to the biotin-lacking cells during stress. These experiments strongly suggest that ceramide (the mammalian type) is the cause of death. We are in the process of determining what sphingolipids are induced in vivo by the combined heat and carbon stress that might be responsible for signaling cellular death in *Neurospora*. Our first approach has been to radiolabel lipids being synthesized under various normal and stress conditions and to separate the component lipids by thin layer chromatography. We have also added inhibitors of de novo sphingolipid biosynthesis to the cells to help identify these molecules.

46. Molecular and functional characterization of *spk1* encoding a putative sphingosine kinase of *Fusarium oxysporum*. Rafael C. Prados-Rosales and Antonio Di Pietro. Departamento de Genetica, Universidad de Cordoba, Campus de Rabanales Ed. C5, 14071 Cordoba, Spain

Sphingolipid long chain bases are important eukaryotic signalling molecules that control vital cellular processes such as proliferation, differentiation and death. In mammalian systems, sphingosine kinase, the enzyme that converts sphingosine to the phosphorylated derivative sphingosine 1-phosphate (S1P), has been implicated in the activation of the extracellular signal-regulated kinase (ERK) MAPK cascade. The orthologous Pmk1-type MAPK cascade in fungi is required for plant infection in a large number of biologically diverse pathogens. At present, the signals and upstream components that activate the Pmk1 pathway during infection are largely unknown. We have initiated a study on the possible role of sphingosine kinase in the activation of Fmk1, a Pmk1-type MAPK from the vascular wilt fungus *Fusarium oxysporum* that is required for root penetration and invasive growth on plant tissue. To this end, we have cloned the *F. oxysporum spk1* gene, encoding a putative polypeptide with homology to sphingosine kinases of yeast and humans, and have constructed an *spk1* allele disrupted by the hygromycin B resistance marker. Knockout mutants are being generated to study the role of Spk1 in signalling and pathogenicity of *F. oxysporum*.

47. Web-based illustrations of the *Neurospora* sexual biology. Namboori B. Raju, Stanford University.

The newly crafted Perkins lab website at Stanford University provides useful resources for *Neurospora* biologists, as well as general mycologists and students. The resources include a photo collection covering: a) meiosis and ascospore development in wild type and mutants of *Neurospora crassa* and *N. tetrasperma*, b) Spore killers, c) chromosome rearrangements, d) meiotic silencing, e) visualization of gene expression using GFP-tagged genes, and f) programmed ascospore death in *Coniochaeta tetraspora*. The photo series are organized into various chapters, each with a summary description and captioned illustrations. The photo archive is accessible through the internet (http://stanford.edu/group/neurospora), and high-resolution versions of these photos are available for educational purposes (not for profit) upon request from the author. The website also includes a complete list of publications from the Perkins lab since 1949, with PDF files of many recent papers and reviews. David Jacobson's help in creating the web site is greatly appreciated. (Support: MCB 0417282 from NSF.)

48. Visualization of meiotic silencing by unpaired DNA (MSUD) using GFP-tagged *histone H1* and *ß-tubulin* in *Neurospora crassa*. Namboori B. Raju[1], Michael Freitag[2], and Robert L. Metzenberg[3]. [1]Stanford Univ., [2]Univ. Oregon, and [3]UCLA.

Shiu *et al.* (2001, *Cell* 107:905) discovered that genes (e.g. *actin, ß-tubulin, mei-3*) inserted into *N. crassa* genome at an ectopic location are not expressed during meiosis in heterozygous crosses where they are unpaired; they coined the term "meiotic silencing by unpaired DNA" (MSUD). With GFP-tagged genes, we can not only localize the gene product, but also observe the expression of MSUD by examining developing asci (Freitag *et al.* 2004, *FGB* 41:897). We used *histone H1-GFP* and *ß-tubulin-GFP* gene inserts at *his-3*. -- When heterozygous, *hH1-GFP* was silenced during ascus development, but only until the time of ascospore delimitation, when it again became active, and the nuclei in four of the eight ascospores fluoresced during development and maturation. In contrast, *hH1-GFP* was not silenced in homozygous asci, where the nuclei glowed throughout ascus development. *ß-tubulin-GFP* was also silenced when heterozygous, but not when homozygous. -- RNA-dependent RNA polymerase is required for meiotic silencing. Mutations in *sad-1*, which lacks the enzyme, suppress meiotic silencing in a semidominant manner (Shiu and Metzenberg 2002, *Genetics* 161:1483). In crosses of *hH1-GFP* and *ß-tubulin-GFP* with *Sad-1*, both *hH1-GFP* and *ß-tubulin-GFP* are expressed throughout ascus development. Spore killers *Sk-2* and *Sk-3* also act as suppressors of MSUD, similar to *Sad-1*. (Support: MCB 0417282 and MCB 0131383 from NSF.)

49. The suppression of meiotic silencing by Spore killers *Sk-2* and *Sk-3* in *Neurospora crassa*. N. B. Raju[1], P. K.T. Shiu[2] and R. L. Metzenberg[2]. [1]Stanford Univ., [2]UCLA.

When genes that are required for meiosis are inserted at an ectopic location, they are not expressed in heterozygous crosses because of meiotic silencing by unpaired DNA (MSUD); consequently, ascus development is abnormal. RNA-dependent RNA polymerase is required for MSUD, and mutations in *sad-1*, which lack the enzyme, suppress MSUD in a semidominant manner (Shiu *et al.* 2001; Shiu and Metzenberg 2002). – Spore killers ($Sk\text{-}2^K$ or $Sk\text{-}3^K$), when crossed with wild type (Sk^S), cause the death of four of the eight ascospores that do not contain the killer (Turner and Perkins 1979). We report here that *Sk-2* and *Sk-3* resemble *Sad-1*, a suppressor of MSUD, in suppressing the silencing of various ectopically inserted genes (*Actin, ß-tubulin, Mei-3, hH1-GFP* and *ß-tubulin-GFP*). Meiotic silencing of *Asm-1* is also suppressed but only partially, and *Round spore* is not suppressed at all. We have demonstrated the suppression of meiotic silencing visually by observing developing asci in Sk^K x *hH1-GFP* and Sk^K x *ß-tubulin-GFP*. Both *hH1-GFP* and *ß-tubulin-GFP* were expressed during meiosis, and all eight young ascospores showed expression of GFP. As the asci matured, the four Sk^S ascospores aborted and degenerated. The remaining four Sk^K ascospores developed normally, with or without the GFP glow, depending upon the segregation of *Sk* and GFP-tagged genes, which are unlinked. Suppression of MSUD by *Sk-2* or *Sk-3* does not depend upon spore killing, however. Although both *Sad-1* and the two Spore killers suppress MSUD, they differ significantly from one another: *Sad-1* is homozygous barren, where as *Sk-2* and *Sk-3* are both homozygous fertile. (Support: MCB 0417282.)

50. A new strategy for identifying cell wall biosynthetic genes in *Aspergillus niger*. R.A. Damveld[1], M. Arentshorst[1], P.A. vanKuyk[1], F.M. Klis[2], C.A.M.J.J. van den Hondel[1,3], A.F.J. Ram[1,3*]. [1]Leiden University, Institute of Biology Leiden, Fungal Genetics Research Group, Leiden, The Netherlands, [2]University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, The Netherlands. [3]Department of Microbiology, TNO Nutrition and Food Research, Zeist, The Netherlands.

The fungal cell wall is an essential organelle. We are interested in identifying new target genes involved in cell wall formation for the development of new antifungal compounds. We found that the expression levels of several genes involved in cell wall biosynthesis were heavily up-regulated when the cell wall integrity of germlings was compromised by the addition of Calcofluor White (CFW). Northern analysis indicated that the mRNA levels of *agsA*, encoding an alpha-1,3-glucan synthase, and *gfaA*, encoding an glutamine:fructose-6-phosphate amino-transferase involved in de biosynthesis of UDP-N-acetyl-glucosamine, were induced 20-fold and 4-fold, respectively. Both genes including at least 2-kb of their respective promoter sequences were cloned. Subsequently, we designed a genetic screen for the isolation of cell wall mutants by cloning the *agsA* promoter region in front of a selectable marker (AmdS). The rationale of this screen is that a mutation in a cell wall biosynthetic gene is expected to result in cell wall weakening and as a result will trigger the expression of the *amdS* gene from the *agsA* promoter, allowing growth on acetamide as the sole N-source. Two hundred and forty mutants were isolated that were able to grow on acetamide. Thirteen of these showed an osmotic-remediable, temperature-sensitive growth defect at 37˚C. Complementation of the mutants and identification of the genes involved is expected to lead to the discovery of new antifungal targets related to cell wall biosynthesis.

51. Characterisation of CwpA, a putative glycosylphosphatidylinositol anchored cell wall mannoprotein in the filamentous fungus *Aspergillus niger*. R.A. Damveld[1], M. Arentshorst[1], P.A. vanKuyk[1], F.M. Klis[2], C.A.M.J.J. van den Hondel[1,3], A.F.J. Ram[1,3*]. [1]Leiden University, Institute of Biology Leiden, Fungal Genetics Research Group, Leiden, The Netherlands, [2]University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, The Netherlands. [3]Department of Microbiology, TNO Nutrition and Food Research, Zeist, The Netherlands.

In fungi, glycosylphosphatidylinositol (GPI)–anchored proteins are found at the cell surface, either attached to the plasma membrane (GPI-PMPs) or attached via a remnant of the GPI-anchor to the cell wall. In the yeasts *S. cerevisiae and C. albicans*, it has been shown that GPI-anchored cell wall mannoproteins (GPI-CWPs) become attached to the beta-1,3-glucan or chitin part of the cell wall through a flexible beta-1,6-glucan moiety. GPI-CWPs can be extracted from the fungal cell wall by treatment with hydrofluoric acid (HF), which cleaves the phosphodiester bonds which is present in the remnant of the GPI-anchor. We show that *A. niger* contains at least five HF-extractable cell wall mannoproteins. A gene encoding an HF-extractable cell wall mannoproteins was cloned and named *cwpA*. The protein sequence of CwpA indicated the presence of two hydrophobic signal sequences at the N-terminus and C-terminus of the protein, for entering the ER and the addition a GPI-anchor, respectively. A CwpA specific antiserum was raised and in combination with simple fractionation experiments, we confirmed that this protein was hardly present in the membrane fraction and abundantly present as a HF-extractable protein in the cell walls. Deletion of *cwpA* did not show an apparent difference in growth under normal growth conditions. The *cwpA* deletion strain displayed however a more sensitive phenotype towards the cell wall disturbing compound Calcofluor White. The latter result indicate the CwpA might have a structural role in maintaining the integrity of the cell wall and that the absence of CwpA leads to weakening of the cell wall which makes the fungal more sensitive to Calcofluor White.

52. A Rho-type GTPase, *rho-4*, is required for septation in *Neurospora crassa*. Carolyn G. Rasmussen and N. Louise Glass. Department of Plant and Microbial Biology. 111 Koshland Hall, University of California, Berkeley, CA 94720-3102

Proteins in the Rho family are small monomeric GTPases primarily involved in polarization, control of cell division and reorganization of cytoskeletal elements. Phylogenetic analysis of predicted fungal Rho proteins suggests that a new Rho-type GTPase family, whose founding member is Rho4 from the archiascomycete *Schizosaccharomyces pombe*, is involved in septation. *S. pombe delta-rho4* mutants have multiple, abnormal septa. In contrast to *S. pombe delta-rho4* mutants, we show that *rho-4* loss-of-function mutants in the filamentous fungus *Neurospora crassa* lead to a loss of septation. Both epitope and GFP-tagged RHO-4 were targeted to septa and to the plasma membrane. RHO-4 formed a ring at incipient septation sites that appeared to constrict with the formation of the septum. RHO-4 remained at mature septa and formed a ring around the septal pore. In other fungi, the steps required for septation include formin, septin and actin localization followed by cell wall synthesis and the completion of septation. *rho-4* mutants were unable to form actin rings, suggesting that RHO-4 acts upstream of actin localization. Further, the maintenance of RHO-4 localization was independent of an intact actin cytoskeleton or microtubule cytoskeleton. Characterization of activated alleles of *rho-4* showed that RHO-4-GTP is likely to initiate new septum formation.

53 Cell biology of conidial anastomosis tubes in *Neurospora crassa*. M.G. Roca, J. Arlt, C.E. Jeffree and N.D. Read. Institute of Cell Biology/COSMIC, University of Edinburgh, Edinburgh EH9 3JH, UK

Although hyphal fusion has been well documented in mature colonies of filamentous fungi, it has been little studied during colony establishment. Here we show that specialized hyphae, called conidial anastomosis tubes (CATs), are produced by all types of conidia and by conidial germ tubes of *Neurospora crassa*. The CAT is shown to be a cellular element that is morphologically and physiologically distinct from a germ tube, and under separate genetic control. In contrast to germ tubes, CATs are thinner, shorter, lack branches, exhibit determinate growth and home towards each other. Evidence for an extracellular CAT inducer derived from conidia was obtained because CAT formation was reduced at low conidial concentrations. A *cr-1* mutant lacking cAMP produced CATs indicating that the inducer is not cAMP. Evidence that the transduction of the CAT inducer signal involves a putative transmembrane protein (HAM-2) and the MAK-2 and NRC-1 proteins of a MAP kinase signaling pathway was obtained because *ham-2, mak-2* and *nrc-1* mutants lacked CATs. Optical tweezers were used as a novel experimental assay to micromanipulate whole conidia and germlings to analyze chemoattraction between CATs during homing. Strains of the same and opposite mating type were shown to home towards each other. The *cr-1* mutant also underwent normal homing indicating that cAMP is not the chemoattractant. Fusion between CATs of opposite mating type was partially inhibited providing evidence of non-self recognition prior to fusion.

54. Live imaging of the secretory pathway in hyphae of *Neurospora crassa*. Meritxell Riquelme[1], Michael Freitag[2], Eddy Sánchez León-Hing[1] and Barry Bowman[3]. [1]Department of Microbiology. Center for Scientific Research and Higher Education of Ensenada. Baja California. México; [2]Institute of Molecular Biology, University of Oregon. Eugene; and [3]Department of Molecular, Cell & Developmental Biology. University of California, Santa Cruz.

Fungal hyphae elongate by apical growth, a complex process that involves the polarized traffic of organelles to the cell apex, and the exocytosis of vesicles in areas of active cell growth. One of the unanswered questions about the establishment of fungal polarity is how vesicles are formed and sorted from sites of synthesis to sites of exocytosis at the plasma membrane. Knowing the correct localization and traffic routes of membrane proteins is essential to better understand how the secretory machinery operates in polarized hyphae. As part of an ongoing project to characterize the organization of the secretory pathway in filamentous fungi, we have set out to localize the plasma membrane H^+-translocating ATPase, a protein carried by secretory vesicles from their point of synthesis in the ER to their destination, along hyphae of *Neurospora crassa*.
We have fused the H^+-ATPase encoding gene *pma-1* from *N. crassa*, with *gfp* and studied its expression in living hyphae of *N. crassa*. Transformants showing positive fluorescence were selected and analyzed by confocal microscopy. The GFP labeled H^+-ATPase was found at the septa and the cell surface of distal parts of the hyphae but not at the apex. Our results confirm earlier predictions (C. L. Slayman, Yale Univ.) that the H^+-ATPase is deficient or inactive at the apex but abundant distally, and provide insight into one of the different pathways for the delivery of proteins from Golgi to the cell surface.

55. Characterization of the *Neurospora crassa* DNA-repair mutants, *mus-43* and *mus-44*. Masahito Sato, Takeru Toko, Takaharu Niki, Yasunori Kato, Masashi Tani, Shintaro Amano, Akihiko Ichiishi. Dept of Life Sciences, TOYO University, Itakura, Gunma, Japan

We previously identified the *mus-43* gene, which was the homolog of human *XPA* and *Saccharomyces cerevisiae RAD14*, and the *mus-44* gene, which was the homolog of human *ERCC1* and *S. cerevisiae RAD10*. Using the repeat-induced point mutation (RIP), we isolated the mutants of those genes. Those mutants were sensitive both to UV and 4-NQO, but not sensitive to MMS. The *mus-44* mutant showed the same sensitivity to UV as the *mus-38*, the previously isolated NER mutant. While the sensitivity of the *mus-43* mutant to UV were milder than the *mus-38* or *mus-44*. To confirm thatthose genes were exclusively involved in NER, epistasis were tested for other repair genes. The four genes, the NER gene *mus-38*, the post-replication repair gene *uvs-2*, the recombination repair gene *mei-3*, and the second excision repair gene *mus-18*, were selected for this purpose. The *mus-38 mus-43* and the *mus-38 mus-44* double mutants showed the same UV-sensitivity as the *mus-38* mutant, while the other double mutants were more sensitive to UV than the parental single mutants.¡¡These results indicate that the *mus-43* and the *mus-44* are components of NER and not involved in other repair systems.
The *mus-43* and *mus-44* mutants were analyzed whether it had the ability to remove CPDs using the *Micrococcus luteus* endonuclease specific to CPDs. These mutants did not show the ability to remove CPDs from the damaged DNA. The two mutants were also analyzed by ELISA using the antibodies specific to CPDs or TC(6-4) photoproducts.

56. *Geosiphon pyriformis* as a model organism for the arbuscular mykorrhiza. Arthur Schuessler and Holger Martin. Darmstadt University of Technology, Institute of Botany, Darmstadt, Germany.

Geosiphon pyriformis is the only known fungus forming endosymbiosis with cyanobacteria. Together with the arbuscular mycorrhizal (AM) fungi, forming AM with the majority of land plants, Geosiphon belongs to the Glomeromycota. The Geosiphon-Nostoc association therefore represents a symbiosis of an ‘AM fungus' with a photoautotrophic prokaryote. Due to this unique life style, the investigations of Geosiphon offers some fundamental advantages when compared to AM. E.g., for gene expression studies, the fungal poly-A mRNA can be easily isolated. Therefore, we use Geosiphon for the identification of 'AM fungal' genes. In the Geosiphon/Nostoc and AM symbioses, the symbiotic interface is the site of nutrient exchange. Via the perisymbiotic membrane the mycobiont receives large amounts of carbohydrates from the photobiont and specific fungal nutrient-transporters are expected to exist. Methods were established to reproducibly isolate fungal mRNA from small samples. After RT, cDNA was amplified by SMART-LD-PCR, size fractionated, ligated in a yeast expression vector, and *E. coli* cells were transformed to construct an expression library.
Since we try to characterize transporter genes involved in nutrient exchange, we used the library for functional complementation of yeast mutants deficient for uptake of amino acids or hexoses. A yeast mutant deficient in hexose uptake was functionally complemented and results about the characterization of the respective gene will be shown.

57. Induction of Apoptosis in *Aspergillus Nidulans* by the *Candida Albicans* Quorum- sensing Molecule Farnesol. Camile P. Semighini, Kenneth W. Nickerson [1] and Steven D. Harris. Plant Science Initiative, [1] School of Biological Sciences, University of Nebraska Lincoln

Candida albicans produces farnesol as an extracellular quorum-sensing molecule. After accumulation above a threshold level, farnesol prevents the yeast to mycelium transition and also blocks formation of biofilm. To our surprise, at concentrations ranging from 20 mM and 200 mM, farnesol has no apparent effect on hyphal morphogenesis in *Aspergillus nidulans*. Instead, it triggered rapid DNA condensation independent of mitosis, intense DNA fragmentation and exposure of phosphatidylserine. These are morphological features that are characteristic of apoptosis. Moreover, farnesol treatment also induced the accumulation of reactive oxygen species. Farnesol is not produced by *A. nidulans* hyphae during growth in liquid culture media, suggesting that it is not acting as a quorum-sensing molecule to regulate colony morphology. Instead, we propose that *A. nidulans* responds to farnesol produced by other fungal species. Future efforts will address the mechanism of farnesol-induced apoptosis, with the goal of identifying potentially novel targets for the development of anti-fungal compounds.

58. Six localisations for six hydrophobins: differential targeting in *C. fulvum*. Hélène LACROIX and Pietro D SPANU, Imperial College London

Cladosporium fulvum has at least 6 hydrophobins named HCf-1 to -6. We have shown that expression of these genes is differentially regulated. We have now investigated the localisation of the hydrophobins during development. To do this we have created transgenic strains of the fungus that carry hydrophobins with the V5 epitope tag. Antibodies that recognise this tag do not cross react with any other *C. fulvum* epitope, the tag is retained in the mature protein if attached close to the C-terminus and does not interfere with the activity of the hydrophobin for which we observe a phenotype. The V5 tag therefore allows us to localise specifically all the hydrophobins identified in this fungus. Our findings to date show that in axenic culture, not only are the hydrophobins produced at different times, but, most unexpectedly, that they were located in different portions of the mycelium. Thus HCf-1 was found in large patches on the conidia, and on the aerial hyphae that produce the conidia. HCf-2 was in a similar position to HCf-1 but the patches were much less frequent. HCf-4 appeared in small areas both in the mycelium that is submerged in the agar and in the aerial hyphae. HCf-5 was visible only on the basal portion of the aerial hyphae at the time of sporulation, but was not found on the conidia. HCf-6 was secreted by the young hyphae 24 hours after germination and was evident as a layer on the surface of the growth medium, not associated with the fungal structures. We will also present data on the localisation of the hydrophobins as *C. fulvum* infects its host, tomato and then discuss the significance of these findings in relation to the possible structure and function of these ubiquitous fungal proteins.

59. Myosin V and conventional kinesin cooperate in hyphal growth of the corn smut fungus *Ustilago maydis*. Isabel Manns, Daniela Aßmann, Anne Straube[1], Uta Fuchs and Gero Steinberg. Max-Planck-Institut für terrestrische Mikrobiologie, Karl-von-Frisch-Straße, D-35043 Marburg, Germany; [1]Wellcome Trust Centre for Cell Biology, University of Edinburgh, Scotland

Long-distance membrane transport is crucial for function and survival of polarized growing cells, such as neurons and fungal hyphae. While evidence exists for a role of microtubules-based kinesins and F-actin associated myosins in polar cell expansion, their functional interplay is only poorly understood. Here we set out to elucidate the role of motors in polarized hyphal growth of fungus *Ustilago maydis*. In hyphae ~90% of all microtubules plus-ends are directed to the expanding tip. However, out of 8 kinesins only Kin3, a Kif1A-like kinesin, and the conventional kinesin Kin2 are crucial for hyphal growth. Dkin3, Dkin2 and kin2/kin3 hyphae were irregular and much shorter, but were still able to grow polarized. A similar phenotype was found in hyphae deleted in myo5, a class V myosin. Interestingly, cell polarity is lost in Dkin2Dmyo5 double mutant, suggesting that both motors cooperate independently to support polar hyphal growth. However, in Dkin2 and Dmyo5 cells, as well as in Dkin2Dmyo5 double mutants phosphatase secretion was reduced to a similar extend, indicating that Myo5 and Kin2 deliver the same class of secretory vesicles. In addition, the tip localization of Kin2-GFP3 requires F-actin and Myo5, and a YFP-Kin2tail fusion protein moves independently of microtubules in a Myo5/actin-dependent manner, suggesting that conventional kinesin is a cargo of myosin V.

60. Blue light negatively regulates the sexual filamentation via the Cwc1 and Cwc2 proteins in *C. neoformans*. Ying-Ku Lu[1], Kai-Hui Sun[1], and Wei-Chiang Shen [1]. [1]Department of Plant Pathology and Microbiology, National Taiwan University, Taipei 106, Taiwan.

Most organisms are capable of sensing light and make appropriate physiological adjustments via complex mechanisms. We use *Cryptococcus neoformans*, a heterothallic basidiomycetous fungus, as a model system to dissect the molecular mechanisms regulating the light responses. In our studies, we demonstrate that the production of sexual dikaryotic filaments is inhibited by blue light. To reveal the molecular mechanisms of blue light photoresponses in C. neoformans, we have identified and characterized two genes, CWC1 and CWC2, which are homologs of the Neurospora crassa wc-1 and wc-2 genes. Analyses of the features and organization of the putative domains indicate that the functions of Cwc1 and Cwc2 proteins may be evolutionally conserved. Through mutation analyses, both cwc1 and cwc2 mutant strains have shown to be insensitive to light. Furthermore, overexpression of the CWC1 or CWC2 gene requires light activation to inhibit sexual filamentation. Taken together, our findings illustrate that blue light negatively regulates the sexual filamentation via the Cwc1 and Cwc2 proteins in C. neoformans. Models are proposed to describe how blue light responses are regulated by the Cwc proteins in C. neoformans, and research is ongoing to test these hypotheses.

61. The *ClaCWH41* gene is involved in appressorial formation and pathogenicity of *Colletotrichum lagenarium*. Rie Matsui[1], Toshihiko Miyaji[1], Gento Tsuji[1], Tomonori Shiraishi[2], Richard O'Connell[1] and Yasuyuki Kubo[1]. [1]Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto Prefectural University, Kyoto, Japan. [1]Laboratory of Plant Pathology, Faculty of Agriculture, Okayama University, Okayama, Japan.

Colletotrichum lagenarium is the causal agent of anthracnose of cucumber. This fungus forms a heavily melanized infection structure called an appressorium. Recently we isolated the mutant p51 by Restriction Enzyme-Mediated Integration mutagenesis, which showed weak pathogenicity. We rescued a fragment from the disrupted gene in the mutant p51. The rescued fragment showed homology with the *CWH41* gene of *Saccharomyces cerevisiae*. The *CWH41* gene encodes alpha-glucosidase and is involved in cell wall alpha-1,6-glucan biosynthesis. We designated the disrupted gene as *ClaCWH41*. To define the character of *ClaCWH41*, we constructed *ClaCWH41* gene disruption mutants. The *clacwh41* mutants were weakly pathogenic on host plants and showed decreased ability to penetrate artificial cellulose membranes. The colony morphology of *clacwh41* mutants was normal, but hyphal growth rates were slightly reduced compared to the wild-type. The most clear difference was observed in appressorium development on glass slide. The *clacwh41* mutants formed abnormally shaped appressoria. These results indicate that the *ClaCWH41* gene plays a key role in appressorium development.

62. Live-cell imaging of the secretory pathway in Trichoderma reesei. Mari Valkonen[1,2], Merja Penttilä[1], Markku Saloheimo[1] and Nick D. Read[2]. [1]VTT Biotechnology, P.O. Box 1500, FIN-02044 VTT, Finland. [2]Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JH, UK

Available data suggest that the basic principles of protein secretion obtained from studies on *S. cerevisiae* and animal cells also apply to filamentous fungi. Although considerable research has been performed on the secretion process in filamentous fungi, little is known about their secretory machinery.
The transport of proteins between the cellular compartments occurs in membrane-bounded vesicles that bud from the donor and fuse with the acceptor compartment. Membrane bound proteins called SNAREs (Soluble N-ethylaleimide-sensitive factor Attachment protein REceptors) play a key role in membrane fusion. SNAREs have been found to be involved in most membrane fusion events in the cell.
This study describes the cloning and characterization of a putative exocytotic t-SNARE (*sso1*) from *Trichoderma reesei*. We cloned the *T. reesei* homologue of *Neurospora crassa nsyn2*. This protein was labeled with the fluorescent protein YFP and the fusion protein expressed in *T. reesei*. Transformants expressing the fusion protein exhibit a clear labeling of the plasma membrane. The fusion protein is uniformly distributed along the hyphae with no tip-focused gradient.
We are precently labeling the v-SNARE SNCI with mRFP with the aim of analyzing its interaction with the t-SNARE SSOI using fluorescence resonance energy transfer (FRET) imaging

63. Study of cell wall integrity and disturbing antifungal compounds with a novel GFP-based reporter method. Robert Damveld[1], John van Dam [1], Frans Klis[2], C.A.M.J.J. van den Hondel[1,3] and A.F.J. Ram[1,3]. Institute of Biology,Wassenaarseweg 64, 2333 AL Leiden, The Netherlands, [2] Swammerdaam Institute for Life Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam [3]Department of Applied Microbiology and Gene Technology, TNO-Nutrition, 3700 AJ Zeist, The Netherlands.

Activation of the fungal cell wall integrity pathway is a mechanism used by fungi to escape from cell wall threatening conditions. In Aspergillus niger we have previously shown that the *agsA* gene, encoding an á-1,3-glucan synthase submit is transcriptionally activated in response to cell wall stress, and that this response is mediated via a RLMA dependent signal transduction pathway. We have used the *agsA* promoter region to set up a GFP-based reporter system to identify compounds that activate the *agsA* expression by fusing the P*agsA* to the cytosolic GFP or to a nuclear targeted version of GFP. The reporter strains confirmed earlier observation that the *agsA* gene is activated in response to various cell wall disturbing compounds such as CFW, caspofungin and tunicamycin. Other forms of stress (osmotic or oxidative stress) did not activate *agsA* expression, indicating that the induction of *agsA* is cell wall stress specific and not a general stress response. We have used the cell wall stress reporter system to study the effect of 20 known antifungal compounds in relation to cell wall remodeling.

64. Isolation of septal pore caps from basidiomycetous fungi. Kenneth G.A. van Driel[1], A.F. van Peer, H.A.B. Wösten, A.J. Verkleij, W.H. Müller, T. Boekhout. [1]Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

The septal pore cap (SPC) structure covers the dolipore, a septal pore surrounded by a donut-like swelling, in many basidiomycetous fungi. The SPC is a membranous structure associated with endoplasmic reticulum. The morphology of the SPC is diverse within the different phylogenetic groups of basidiomycetes and can be divided in several main categories: vesiculate or tubulate, imperforate, and perforate. Though electron microscopical (EM) studies revealed the SPC in great detail, the function of the SPC is only poorly understood. Our aim was to isolate and enrich SPCs to characterize its proteins and genes that are involved in the formation of the SPC. This will lead to a better understanding of the role of SPCs in basidiomycetous cells .
We successfully enriched SPCs from *Rhizoctonia solani* cell fractions. After EM studies we observed that the plug material at the orifice of the septal pore channel stayed attached via fibrillar material to SPCs. Protein electrophoresis showed that a 18 kDa glycoprotein was enriched in the SPC fraction. This protein was N-terminally sequenced. We raised antibodies against this protein to perform immunolabeling studies. From our observations we think that the SPC may be involved in the production of plugging material. Alternatively, the SPC may function as a repository of the plugging material that can be released upon plugging the septal pore during i.e. stress situations.

65. Complementation of a Calcofluor-hypersensitive mutant in *Aspergillus nidulans* by a novel transmembrane protein. Stanley Vance, Lisa Harsch, Darlene Loprete and Terry Hill. Departments of Biology and Chemistry, Rhodes College, Memphis, TN.

In a search for as-yet-unidentified genes whose function bears upon cell wall integrity, this laboratory is screening strains from the Harris *et al.* collection (Genetics 136:516-532, 1994) of *Aspergillus nidulans* temperature-sensitive morphology mutants, as well as mutant strains of our own creation, using hypersensitivity to the cell wall compromising agent Calcofluor White (CFW) as the primary screening criterion. One such strain, ts1-49, shows hypersensitivity not just to CFW, but also to the unrelated wall-compromising agent Congo Red and to the detergent SDS, when compared to the respective wild type. The strain does not, however, manifest a morphological phenotype at 42C. We have complemented the hypersensitive aspects of the phenotype with two separate plasmids from the Osherov and May (Genetics 155: 647-656, 2000) "AMA *NotI*" genomic DNA library. The overlapping regions of the two plasmids contain a single predicted gene, designated AN2880.2 (*Aspergillus* Sequencing Project, Center for Genome Research). The ca. 114 kDa hypothetical translated product contains a ca. 538 amino acid long internal sequence which is homologous to DUF221 domains found in a variety of multipass transmembrane proteins of unknown function. On-line domain-predicting programs predict eleven membrane-spanning helices, with the N-terminus oriented towards the cell interior. Work is underway to sequence the corresponding region of the mutant (ts1-49) genomic DNA, to determine whether AN2880.2 is the gene mutated in strain ts1-49 or a high-copy suppressor.

66. The role of a spindle pole body associated protein in cytoplasmic microtubule organization. Daniel Veith and Reinhard Fischer. University of Karlsruhe and University of Marburg.

Polarized growth and nuclear migration are key features of both uninucleate yeasts and syncytial filamentous fungi. Microtubules and microtubule-dependent motor proteins are key players, whose arrangement, interaction and contribution to nuclear distribution are in the focus of interest. We have studied an *A. nidulans* mutant, *apsB*, in which nuclear positioning is disturbed and identified ApsB (GFP-ApsB) as a novel spindle-pole body (SPB) associated protein. Interestingly, ApsB not only localises to SPB´s of interphase nuclei and the poles of the mitotic spindle, but unexpectedly, it was also detected at septa. Even more surprisingly, ApsB was transported very fast along microtubule tracks towards the plus- or the minus-end of microtubules. Recently, it was shown that microtubule-organizing centres were localized at nuclei, in the cytoplasm, and at septa. Thus, ApsB appeared to play a role in microtubule formation. In agreement with this, in *apsB* mutants the network of cytoplasmic microtubules as well as the number of astral microtubules was dramatically reduced, explaining the disturbed nuclear distribution pattern.

67. Flo11p, an *S. cerevisiae* flocculin, mediates adhesion to extracellular matrix proteins. S. Venkatraman, D.C. Bartelt and A.M. Dranginis. St. John's University, Dept. Biology, Queens, NY 11439.
The cell wall protein Flo11p is required for a variety of processes that involve adhesion in *S. cerevisiae*, including flocculation, adhesion to plastic and agar substrates, development of pseudohyphae and formation of biofilms. Flo11p is not expressed in the standard laboratory strain of *S.cerevisiae*, S288C, due to a mutation in a transcriptional activator required for its expression. Unlike many fungi, S288C-background yeast is not known to adhere specifically to mammalian extracellular matrix (ECM). We tested the hypothesis that Flo11p is the molecule responsible for ECM binding, by coating wells of microtiter plates with various ECM proteins and measuring the binding of yeast to the wells. *FLO11*-expressing yeast bound to wells coated with the ECM proteins fibronectin and laminin, whereas cells with a mutation in *FLO11* failed to bind under these conditions. Flo11p does not mediate nonspecific binding to all glycoproteins, since no binding was observed to wells coated with carboxypeptidase Y, fetuin or asialofetuin. The binding of Flo11-bearing cells to fibronectin was saturable and dose-dependent. Pre-incubation of the yeast cells with fibronectin resulted in greatly reduced binding, as did pre-incubation of the coated wells with antibodies to fibronectin. The binding of *FLO11*-expressing yeast to ECM proteins thus appears to be specific, and provides a model for the study of adhesion of fungi to human tissue.
Supported by NIH grant 1R15A143927-01 and NSF grant MCB-9973776.

68. Unique roles for *Aspergillus nidulans* Spa2 and Bud6 homologues in polarized growth and septation of hyphae . Aleksandra Virag, Claire Pearson, Steven Harris. Plant Science Initiative, University of Nebraska, Lincoln, NE

Although a growing number of studies address hyphal development in filamentous fungi, how cellular components organize to initiate, maintain and regulate highly polarized hyphal growth is not fully understood. In yeast, a complex termed the polarisome containing Spa2, Pea2 and Bud6, is present at sites of polarized growth, with Bni1 (formin) interacting with both Spa2 and Bud6. These gene products are involved in directing components that are to be incorporated into sites of cell surface expansion to their final destinations. In filamentous fungi, homologues of some polarisome components, such as Bni1, Spa2 and Bud6 are present, while others, such as Pea2, are absent, suggesting a unique role for these genes and gene products in polarized growth of filamentous fungi. In *Aspergillus nidulans* the Bni1 homologue, SepA, is present at sites of hyphal tip growth, as well as sites of septation. We constructed GFP fusions with the *A. nidulans* Bud6 and Spa2 homologues (BudA and SpaA) and looked at the distribution of the fusion proteins. SpaA-GFP localized to the hyphal tip, while the BudA-GFP localized to sites of septum formation. Under repressing conditions, BudA::GFP expressed under the regulation of the *alcA* promoter showed reduced branching and a delay in septation. Our results suggest that both SpaA and BudA function at hyphal tips and branching sites, and that BudA may have an additional function at sites of septum formation. We also present a method that identifies gene products involved in reconstituting a lost polarization axis by assessing the number of hyphae that resume their original direction of growth after depolarizing the tip with cytochalasin A.

69. Initial characterization of a mutation isolated as a suppressor of the col-16 mutation of *N. crassa*. Michael K. Watters and Margaret Munich. Valparaiso University, Valparaiso, IN.

Growth in filamentous fungi occurs at a tip which branches as it extends. The col-16 mutant of *N. crassa* is one of many which displays a greater branching frequency than the wild type and therefore grows more densely. Following UV mutagenesis, colonies were screened for those in which more wild type growth had been restored with the goal of finding suppressors. One of the these suppressed strains (col-16, sup28) was chosen for further characterization. Strains containing the suppressor sup28 separated from col-16 were obtained using unordered tetrads. Examination of the morphology of a sup28 mutant strain shows that while the rate of growth is severely reduced, the basic branching pattern (type of branches as well as distance between branch points) is unaltered. Further characterization of the sup28 mutation suggests that it is a leaky auxotroph.

70. In vivo time lapse microscopy of organelle distribution shows that the WASP family member protein Wal1p is required for vacuolar motility but not for mitochondrial distribution in the filamentous ascomycete Ashbya gossypii. Andrea Walther and Jürgen Wendland. Dept. of Microbiology, Friedrich-Schiller University and Leibniz Institute for Natural Products Research and Infection Biology - Hans-Knoell-Institute, Jena, Germany

During polarized hyphal growth in the Ashbya gossypii wild type cytoplasmic streaming can be visualized as the tipward movement of large vacuoles using differential interference contrast (DIC) imaging methods. Interestingly, the direction of cytoplasmic streaming can be reversed due to either contact inhibition of the hyphal tip that blocks further elongation or cessation of growth at the tip. This suggests that hyphal tips act as a sink for cytoplasmic transport . However, septation blocked the passage of vacuoles and thus restricted the cytoplasm in hyphal compartments.
We have analyzed the requirements of the actin cytoskeleton for these processes and describe the role of the Wiskott-Aldrich Syndrome Protein (WASP)-homolog of A. gossypii encoded by WAL1 involved in endocytosis and vacuolar movement.
Wal1 is required for the maintenance of polarized hyphal growth and wal1 mutants exhibit a drastically decreased hyphal growth rate. In the wal1 mutant strain cortical actin patches are not localized to the hyphal tip but accumulate in subapical regions. This positioning of patches directs fluid phase endocytosis away from the hyphal tip to subapical regions. Loss of WAL1 results in defects in endocytosis, vacuole motility and vacuole movement. In contrast, the tipward localization and redistribution of mitochondria is not dependent on Wal1p.
Our results suggest that Wal1p dependent actin assembly is required for endocytosis and vacuolar movement but that other processes, e.g. formin mediated actin assembly may be involved in mitochondrial distribution.

71. Role of *Neurospora crassa* kinesins. Renate Dombi, Florian Fuchs, Sarah Adio, Benedikt Westermann, Manfred Schliwa, Guenther Woehlke. Adolf-Butenandt-Institute, University Munich, Germany

The genome of *Neurospora crassa* contains genes for 10 kinesin-related proteins, among them 3 putative microtubule-dependent vesicle motors. The conventional kinesin NcKin has been known for several years, and extensively been characterized in vivo and in vitro. Two unconventional kinesins of the kinesin-3 (fromerly Unc104 or Kif1) family are also present, one of which shows similarity to other kinesin-3 motors (NcKin2). The other kinesin (NcKin3) is unusually short and seems to be specific for filamentous fungi.
We cloned the cDNA of NcKin3, and expressed truncated variants of the motor in bacteria. The in vitro ATPase and motility behavior shows that NcKin3 is a dimeric microtubule motor protein that moves at a speed of 0.6 micro-m/s, but apparently by a different mechanism than conventional kinesins.
To investigate the cellular function of both kinesin-3 familiy motors, we knocked out NcKin2 and NcKin3. None of the knock-out strains shows any apparent phenotype, suggesting that *Neurospora* is able to suppress the phenotype under laboratory conditions. Unlike in the NcKin knock-out, the growth rate is normal, and the distribution of organelles is unchanged. To test whether both motors act redundantly, we are in the process of crosssing the knock-out strains, and to generate dominant negative mutants. So far, we were able to detect an up-regulation of NcKin3 in the NcKin2 knock-out strain, but no obvious suppressor for the NcKin3 knock-out using a proteomic approach.
We acknowledge financial support from the Deutsche Forschungsgemeinschaft.

72. Optical Tweezer Micromanipulation of Filamentous Fungi. Graham Wright[1,2], Jochen Arlt[2], M. Gabriela Roca[1,2], Wilson Poon[2,3] & Nick Read[1,2]. [1]Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JH, UK. [2]COSMIC, University of Edinburgh, Edinburgh, EH9 3JZ, UK. [3]School of Physics, University of Edinburgh, Edinburgh, EH9 3JZ, UK

Optical tweezers allow the non-invasive micromanipulation of living cells by utilising the forces generated from the interaction of laser light with the sample. We have designed and built a simple, user friendly, steerable optical tweezer system (using a 785 nm diode laser) to manipulate spores, spore germlings and mature hyphae of the model filamentous fungus, *Neurospora crassa*. More specifically we are: (1) manipulating the so called Spitzenkorper, the behaviour of which is intimately associated with the pattern and direction of tip-growth; (2) using beads to assess and quantify the growing forces of hyphal tips and germlings; (4) disrupting the chemotropic gradients between spore germlings which are growing towards each other by moving one of the participating germlings; and (5) manipulating the Woronin body, an organelle involved in occlusion of septal pores after hyphal damage.

73. The histidine kinase Dic1p is a positive regulator of the Hog1-type MAP kinase in *Cochliobolus heterostrophus.* Akira Yoshimi[1], Kaihei Kojima[2,3], Yoshitaka Takano[2], and Chihiro Tanaka[1]. [1]Laboratory of Environmental Mycoscience, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan, and [2]Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan. [3]Present address: Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, USA.

In Southern corn leaf bright fungus *Cochliobolus heterostrophus*, the histidine kinase Dic1p is involved in resistance to dicarboximide and phenylpyrrole fungicides and osmotic adaptation. We previously reported that the phenylpyrrole fungicide fludioxonil led to improper activation of Hog1-type MAPKs in some phytopathogenic fungi including *C. heterostrophus*. To elucidate the relationship of *C. heterostrophus* BmHog1p (Hog1-type MAPK) and the histidine kinase Dic1p, the phosphorylations of BmHog1p were analyzed in the wild-type and the *dic1* deficient strains by western blotting. In the wild-type strain, the phosphorylated BmHog1p was detected after exposure to both dicarboximide iprodione and phenylpyrrole fludioxonil, even at concentration of 1 µg/ml. In the *dic1* strain, no phosphorylated BmHog1p was detected after exposure to 1 µg/ml and 10 µg/ml of the fungicides. Similarly, in response to osmotic stress (0.4 M KCl), a little amount of phosphorylated BmHog1p was found in the *dic1* strain, whereas the band representing the active BmHog1p was clearly detected in the wild-type strain. Similar results were obtained in the case of Os2p MAPK phosphorylation in the histidine kinase gene *os-1* mutants of *Neurospora crassa*. These results suggest that in general the Dic1/OS1-related histidine kinase is a positive regulator of Hog1-type MAPKs in filamentous fungi. Importantly, the activations of Hog1-type MAPKs were observed at much higher levels of the fungicides (100 µg/ml) and osmotic stress (0.8 M KCl) in these histidine kinase mutants of *C. heterostrophus* and *N. crassa*. This suggests that another signaling pathway activate Hog1-type MAPKs, especially when cells are exposed to high-levels of the fungicides and osmotic stress.

74. Use of RNAi for partial inactivation of the essential COT1 kinase in *Neurospora crassa*. Carmit Ziv, Efrat Dvash and Oded Yarden. Department of Plant Pathology and Microbiology and Otto Warburg Center for Agricultural Biotechnology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel

Neurospora crassa is a useful model organism for studies of cell polarity. The *N. crassa cot-1* gene encodes a serine/threonine protein kinase involved in apical hyphal cell elongation. Disfunction of this kinase leads to a dramatic and pleotropic change in hyphal morphology; however, its actual role has yet to be determined. We adapted RNAi as a silencing mechanism to reduce *cot-1* gene expression to learn its role in the hyphal elongation process. A hairpin construct of the *cot-1* gene was assembled using the first 1800bp of the genomic fragment that includes the *cot-1* promoter and the first two exons. Transformants were screened for altered morphology and were subjected to morphological and molecular characterization. Our results demonstrate that RNAi can be employed as a partial gene silencing mechanism, resulting in a range of different mutants. This wide-range silencing system may enable us to study the different functions of *cot-1* by disassociating some of the multiple defects from each other and reducing the pleiotropy resulting from highly impaired COT1 activity.

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75. Some Like it Hot: differences in Thermotolerance of Coccidioides species
Bridget M. Barker[1,2], Sarah N. Statt[1], John N. Galgiani[2], Marc J. Orbach[1]. [1]Department of Plant Sciences, Division of Plant Pathology and Microbiology, University of Arizona, Tucson, AZ. [2] Southern Arizona Veterans Administration Health Care System, Tucson, AZ.

Recent division of Coccidioides into two species, *C. immitis* and *C. posadasii*, was based on molecular phylogenetic evidence defining two distinct clades, but very little phenotypic difference has been seen between the two species (Fisher et al. 2002). A subtle salt tolerance difference was observed where initial growth was slower for *C. posadasii*, but by two weeks,was not significant. While surveying strains to develop selective conditions for recovery of *C. posadasii* from the desert soil, we have identified a significant difference in growth rate. A subset of 22 *C. posadasii* and 34 *C. immitis* isolates, representing its full geographical range were screened at 37ºC and 28ºC. Radial growth rate was measured over 16 days. We found that *C. posadasii* grew significantly faster at 37ºC, when compared to *C. immitis*. There was no significant difference in growth rate at 28ºC. The division of Coccidioides into two species has caused concern among many people in the clinical setting. Diagnosis of coccidioidomycosis does not require that clinicians know the species that caused the disease, but there are questions about disease severity and treatment. Our research indicates there are differences related to temperature, which is the first significant phenotypic difference between the species and is easily assayable in a clinical setting.

76. The relative contributions of seedborne and windborne inoculum to foliar epidemics of *Phaeosphaeria nodorum*. Rebecca S. Bennett[1], Michael G. Milgroom[1], Barry M. Cunfer[2], and Gary C. Bergstrom[1]. [1]Department of Plant Pathology, Cornell Univ., Ithaca, NY. [2]Department of Plant Pathology, Univ. of Georgia, Griffin, GA.

Population genetic and epidemiological data, when examined separately, have resulted in different hypotheses about the predominant source of inoculum in the *Phaeosphaeria nodorum*-wheat pathosystem, i.e., sexually-derived, windborne ascospores versus asexual, seedborne inoculum, respectively. We are addressing these competing hypotheses by using a mark-recapture experiment in which seedborne isolates of *P. nodorum* can be identified by rare alleles. We planted infected wheat seed in experimental plots and sampled *P. nodorum* throughout the growing season. If inoculum comes primarily from seed, we expect to find that isolates collected have the same rare alleles as in our released isolates. If the inoculum is coming primarily from immigrant, windborne ascospores, we expect to find that isolates collected from different experimental plots are genetically similar to each other but distinct from artificially constructed seed populations. Analyses of early foliar samples indicate that infected seed was likely to be more important that ascospore inoculum. Data from late foliar samples and harvested seed will also be presented.

77. Cross fertility of lineages of *Gibberella zeae*. R.L. Bowden[1], J.F. Leslie[2], Jungkwan Lee[2], and Yin-Won Lee[3]. [1]USDA-ARS Plant Science and Entomology Research Unit, Manhattan, KS; [2]Department of Plant Pathology, Kansas State University, Manhattan, KS; [3]School of Agricultural Biotechnology, Seoul National University, Seoul, Korea

O'Donnell et al. (2000) divided *Gibberella zeae* into seven phylogenetic lineages and these were later extended to nine and given species rank (O'Donnell et al. (2004)). The objective of this study was to estimate the potential for genetic exchange between these lineages by quantifying cross fertility in the laboratory. Crosses were conducted on carrot agar as described by Bowden and Leslie (1999). Three strains of G. zeae lineage 7 with an insertion in the MAT1-2 locus that renders them heterothallic were used as females (Lee et al., 2003). Standardized suspensions of macroconidia from strains of each of the nine lineages were used as males to fertilize the females. Ten days after fertilization, carrot agar plates were inverted over water agar plates and fertility was measured by counting ascospores deposited overnight. Homothallic cultures and unfertilized heterothallic strains served as controls. Cross fertility was highly variable and differed for the three female strains. All males from all lineages produced viable progeny with at least two lineage 7 female strains. Certain pairings of lineage 7 females with males of other lineages showed fertility levels comparable to lineage 7 x 7 crosses. No evidence for consistent fertility barriers between lineage 7 and the others was found.

78. Conservation of non-ribosomal peptide synthetases in closely related Cochliobolus species. Kathryn E. Bushley and B. Gillian Turgeon. Department of Plant Pathology, Cornell University, Ithaca, NY, 14853

Non-ribosomal peptide synthetases (NRPSs) are multimodular enzymes, found in ascomycete fungi and bacteria that make peptides (NRPs) through a thiotemplate mechanism independent of ribosomes. NRPs are structurally diverse and have a broad spectrum of biological activities, both beneficial and harmful. A minimal NRPS module, encoded by a non-ribosomal peptide synthetase gene (NPS), is composed of an adenylation (AMP) and a thiolation domain. A condensation domain functions in peptide bond formation and elongation of the nascent peptide. Previous studies suggest that genes encoding NRPSs have a discontinuous distribution in the genomes of distantly related filamentous ascomycetes (Kroken et al, unpublished). Various evolutionary processes could explain this pattern: 1) gene duplication and differential loss, 2) recombination 3) gene conversion, and 4) horizontal gene transfer. We are investigating diversity and evolutionary history of NPSs among closely related taxa, using three Cochliobolus species (C. heterostrophus (Ch), C. carbonum (Cc), and C. victoriae (Cv) to address which mechanism(s) may explain the diversity across divergent taxa. For this, sequence data from conserved motifs of 25 AMP domains, extracted from 11 NPSs in identified in Ch (Lee et al, in press), were used to design degenerate and specific primers to identify counterparts of the known ChNPSs, as well as previously unidentified NPSs, in Cv and Cc. Results suggest that the set of ChAMP domains is largely conserved in Cv and Cc, however differences exist. Six previously unidentified Cv- and one Cc-unique AMP domain have been identified, while attempts to amplify ChNPS8 and ChNPS11 AMP domains and one ChNPS9 AMP domain have failed. Current work focuses on whether AMP domains within each Chgene are syntenous across species. Initial results suggest that the order of AMP domains in at least two genes, NPS1 and NPS2, is conserved across species. These data will allow us to evaluate whether recombination plays a role in generating new NPSs.

79. The Evolutionary Genetics of Dikaryosis Versus Diploidy. Travis A. Clark and James B. Anderson. Department of Botany, University of Toronto, Ontario, Canada.

While many eukaryotic organisms exist as diploids, with the two gametic genomes residing in the same nucleus, most basidiomycete fungi exist as dikaryons, with the two gametic genomes residing in separate nuclei that are physically paired. To determine if haploid monokaryotic and dikaryotic mycelia of *Schizophyllum commune* adapt to novel environments under natural selection, we serially transferred replicate populations of each ploidy state on minimal medium for 24 months (17,500 generations). Dikaryotic mycelia responded to selection with increases in growth rate, while haploid monokaryotic mycelia did not. To determine if the haploid components of the dikaryon adapt reciprocally to one another's presence over time, we recovered the intact haploid components of dikaryotic mycelia at different time points (without meiosis) and mated them with nuclei of different evolutionary histories. We found evidence for coadaptation between nuclei in one dikaryotic line, in which a dominant deleterious mutation in one nucleus was followed by a compensatory mutation in the other nucleus; the mutant nuclei that evolved together had the best overall fitness. In other lines, nuclei had equal or higher fitness when paired with nuclei of other histories, indicating a heterozygote advantage. To determine if genetic exchange occurs between the two nuclei of a dikaryon, we developed a 24-locus genotyping system based on single nucleotide polymorphisms to monitor somatic exchange. We observed genetic exchange and recombination between the nuclei of several different dikaryons, resulting in genotypic variation in these mitotic cell lineages. We are currently conducting experiments to compare the adaptive potential of the dikaryotic state with that of the corresponding, isogenic diploid state. The hypothesis is that dikaryons and diploids differ with respect to their patterns of genetic change over time, with accompanying differences in the patterns of gene regulation and phenotypic plasticity.

80. Diversity in natural populations of *Colletotrichum* from the tallgrass prairie. Jo Anne Crouch, Bruce B. Clarke, and Bradley I. Hillman. Department of Plant Biology and Pathology, Rutgers University.

The fungal genus *Colletotrichum* contains several species infecting monocot hosts in both cultivated and natural grass communities. In monocultured agroecosystems, these fungi are often found as destructive pathogens, capable of inducing significant disease in the host plant. In contrast, our recent sampling of *Colletotrichum* in a natural tallgrass prairie ecosystem suggests that populations of this fungus living in diverse grass communities maintain a non-pathogenic lifestyle, with their presence never correlated with substantial disease. Preliminary multi-locus phylogenetic analysis supports the presence of a single lineage of *Colletotrichum* in the grassland environment, but transposon distribution data and RFLP patterns suggest that these populations may actually represent a hybrid zone between distinct *Colletotrichum* phylogenetic species. We are currently employing microsatellite markers to evaluate whether natural grasslands represent regions of hybridization for *Colletotrichum* species that inhabit Pooideae grasses.

81. Evolutionary relationships of *Colletotrichum* species pathogenic to grasses of the Poaceae. Jo Anne Crouch, Bruce B. Clarke, and Bradley I. Hillman. Rutgers University, New Brunswick, NJ

Colletotrichum graminicola is a filamentous, primarily clonally-reproducing fungus inhabiting a wide range of grasses and cereals. During the past several years, this fungus has emerged as one of the most important pathogens in turfgrass systems. Control of the disease is often unpredictable, possibly due to an imperfect understanding of population structure and pathogen variability. Using Bayesian likelihood analyses of nucleotide sequences from three unlinked nuclear loci, we provide compelling evidence of sympatric, species-level phylogenetic divergence between *C. graminicola* pathogenic to *Zea mays* and *Colletotrichum* specimens causing disease in grasses of the subfamily Pooideae. These data also revealed two lineages emerging along distinct evolutionary trajectories among isolates sampled from Pooideae grasses, including several Pooid species cultivated as turfgrasses. Nucleotide substitution rates consistent with positive adaptive selection at the fungal mating-type gene suggests that reinforcement may have played a role in reproductively isolating these unique sibling species as they emerged in host-range restricted ecological niches. Transposon distribution patterns and RFLP fingerprint analysis extend the phylogenetic estimations, independently supporting the conclusion that several closely related cryptic species of *Colleotrichum* inhabit the grasses of North America.

82. Het-S spore killing in *Podospora anserina*: Non-Mendelian nuclear inheritance caused by the non-Mendelian cytoplasmic HET-s prion-element. Henk Dalstra, Klaas Swart, Sven Saupe and Fons Debets. Laboratory of Genetics, Wageningen, The Netherlands

Two alleles of the *het-s/S* locus occur naturally in *Podospora anserina*, *het-s* and *het-S*. The *het-s* encoded protein can form a prion that propagates a self-perpetuating amyloid aggregate, resulting in two phenotypes for the *het-s* strains. The prion infected [Het-s] shows an antagonistic interaction to *het-S* whereas the prion-free [Het-s*] is neutral in interaction to *het-S*. The antagonism between [Het-s] and *het-S* is seen as heterokaryon incompatibility at the somatic level and as *het-S* spore killing in the sexual cycle. The consequences of the unique transition from a coenocytic to a cellular state in the sexual phase and the timing and localization of paternal and maternal HET-s and HET-S expression that are pertinent to prion transmission and *het-S* spore killing are elaborated. A model for *het-S* spore killing is proposed.

83. Experimental Evolution of Reproductive Isolation in Neurospora. Jeremy Dettman, James Anderson, and Linda Kohn. Department of Botany, University of Toronto, Ontario, Canada

Reproductive isolation may develop between populations by genetic drift alone, or as a by-product of adaptation to divergent environmental conditions (ecological speciation). Interspecific hybridization may also produce novel combinations of alleles which may facilitate speciation by creating new opportunities for adaptive evolution (hybrid speciation). The evolution of reproductive isolation typically occurs over long time scales and is difficult to observe directly in nature. To study these modes of speciation, we are experimentally evolving populations of Neurospora in controlled environments and are monitoring changes in reproductive behavior as they occur. Populations of *N. crassa* and *N. crassa-N. intermedia* hybrids are being serially propagated asexually, with periodic sexual cycles to maintain the capability for sexual reproduction within lineages. To promote divergent adaptation, replicate lineages are being evolved under two different suboptimal growth conditions (high salinity and low temperature). At the end of the evolution regime, individuals from the evolved lineages will be mated and reproductive success will be measured. Several questions will be addressed: Do the populations adapt to the novel environments? Are lineages evolved in divergent environments more likely to develop reproduction isolation than lineages evolved in the same environment? Does the increased genetic diversity of hybrid lineages allow for more rapid development of reproductive isolation among hybrid lineages? Do similar phenotypes of reproductive isolation develop between hybrid lineage pairs and non-hybrid lineage pairs? Preliminary tests after two months of evolution indicate that some forms of reproductive defects have arisen. Results of these and additional tests will be presented and discussed.

84. Landscape genetics of *Penicillium marneffei* in southeast Asia. Matthew C. Fisher Dpt. Infectious Disease, Imperial College London, UK

High-throughput multilocus sequence typing (MLST) and multilocus microsatellite typing (MLMT) techniques are uncovering a wealth of genetic diversity hidden within morphologically-defined fungal species. New statistical tools (such as Bayesian approaches) are enabling us to analyse the structure of this genetic diversity within species; these analyses are then allowing us to understand the microevolutionary processes that are generating genetic structure. MLMT of Penicillium marneffei has shown the existence of high levels of genetic diversity; visualizing this diversity within a spatial context shows that landform impacts strongly on genetic structure. Despite the high dispersive capacity of fungal spores, gene-flow in P. marneffei appear to be low, suggesting that microevolution of isolates is causing local adaptation to their immediate environment. In order to test this hypothesis, highly sensitive environmental PCRs are being used to detect P. marneffei in the environment. Remotely-sensed GIS databases are then being used to correlate the presence/absence of the fungus with specific environmental variables in order to determine the ecotypes within which P. marneffei is associated. It is then possible to use MLMT data to determine the extent to which P. marneffei genetic structure is determined by landform and ecotype.

85. Convergent evolution of chromosomal sex-determining regions in the animal, plant and fungal kingdoms. James A. Fraser,

Stephanie Diezmann, Ryan L. Subaran, Andria Allen, Klaus B. Lengeler, Fred S. Dietrich, and Joseph Heitman. Duke University Medical Center

Sexual identity is governed by sex chromosomes in plants and animals, and mating type loci (MAT) in fungi. Comparative analysis of the MAT locus from a species cluster of the human fungal pathogen *Cryptococcus* revealed sequential evolutionary events that fashioned this large, highly unusual region. We hypothesize MAT evolved via four main steps beginning with gene acquisition into two unlinked sex-determining regions, forming independent gene clusters involved in pheromone production/sensing and meiosis/karyogamy. These cluster then fused via chromosomal translocation. The transitional tripolar intermediate state created was then converted to a bipolar system via gene conversion or recombination between the linked and unlinked sex determining regions. Experimental tests of this model are currently in progress. MAT was subsequently subjected to intra- and interallelic gene conversion and inversions that suppress recombination. Large-scale analysis of MAT structure of >100 isolates revealed a major force driving this evolution was the acquisition of transposable elements, with the locus transposon content over 4 times greater than elsewhere in the genome. These events resemble those that shaped mammalian and plant sex chromosomes, illustrating convergent evolution in sex-determining structures in the animal, plant and fungal kingdoms.

86. *Fusarium graminearum* in the U.S.: heterogeneous and in flux. Liane Rosewich Gale[1], Je'Nise D. Bryant[2], Gerald E. Ochocki[1], Todd J. Ward[3], and H. Corby Kistler[1]. [1]USDA, CDL, St. Paul, MN. [2]University of Minnesota, St. Paul, MN. [3]USDA, NCAUR, Peoria, IL.

From a previous survey of diversity in *Fusarium graminearum* collected from 86 fields in 53 counties in 9 midwestern states in 1999 and 2000, we identified a small population in ND and MN (7%) that produced 3-acetyl, deoxynivalenol (3ADON) that were genetically distinct (Nm = 0.5) from the predominant 15-acetyl, deoxynivalenol (15ADON) producing *F. graminearum*. Collections in 2003 from 40 wheat fields in 24 counties in ND, and 9 fields in 7 counties in MN resulted in 2,133 isolates. The 3ADON type was widespread and at high frequency (21% in ND, 24% in MN). Further analysis with primers targeting three VNTR loci demonstrated that recombination in *F. graminearum*, although occurring, may be an infrequent event, as only 70 potential recombinants between the two populations were identified. Chemotyping in collections from 2001-2003 indicates that 15ADON is still the only type in other midwestern states, though the nivalenol type was the most frequent in isolates from LA. The predominance of the nivalenol type in LA and the build-up of the 3ADON type in MN and ND suggest that selection is a principal evolutionary force acting on populations of *F. graminearum*. At the same time this study demonstrates the need for continual monitoring of the population composition, as *F. graminearum* in the U.S. is certainly not as homogeneous as previously believed.

87. Association of clinical, environmental and plant pathogenic isolates within the *Fusarium solani* species complex. Ning Zhang[1], Kerry O'Donnell[2], and David M. Geiser[1]. [1]Department of Plant Pathology, Penn State University, University Park, PA USA. [2] MGBRU/NCAUR/ARS/USDA, Peoria, IL USA

Members of the *Fusarium solani* species complex are increasingly implicated as the causative agents of human mycoses, particularly in the immunocompromised patient population. Previously members of this complex were shown to comprise at least 26 phylogenetically distinct species, including ubiquitous saprophytes and economically important plant pathogens. To identify phylogenetic species associated with human infections, we generated three partial gene sequences (1726 bp) from 471 isolates from clinical (human N= 278, hospital environment N =21) and non-clinical (N = 172) sources chosen to represent the known phylogenetic diversity in the complex. We identified 218 four locus haplotypes among these isolates, 99 of which included clinical isolates. Nineteen haplotypes included both clinical and non-clinical isolates, and monophyly of the clinical isolates was rejected. Members of the most common environmental haplotypes, including those found in hospitals, were associated with the majority of *F. solani* infections, suggesting that *F. solani* infections are truly opportunistic across a wide range of phylogenetic species and haplotype diversity. The majority (77%) of clinical isolates fell into one of four clades of haplotypes, each of which included isolates from non-clinical sources. One of these clades corresponded to Mating Population V of the *F. solani* species complex.

88. Improved bioinformatic tools for identification of fungi from environmental samples based on ITS and LSU rDNA sequences. József Geml, James Long, Shawn Houston, Thomas G. Marr, D. Lee Taylor. Institute of Arctic Biology, University of Alaska Fairbanks, 311 Irving I, Fairbanks, AK, 99775, U.S.A.

To study the fungal diversity in boreal forests of Alaska we are sequencing 30,000 clones from PCR libraries of fungal ribosomal gene-regions amplified from soil DNA extracts. In addition, we will compare sporocarp versus soil views of fungal diversity by sequencing 4000 sporocarps from the UAF Mycological Herbarium. Database similarity searches play crucial role in matching unknown environmental sequences to sequences from identified fruitbodies. In this paper, we assess the performance of different similarity search algorithms (BLASTN, FASTA) - with and without masking highly conserved regions - against newly assembled databases of fungal ITS and LSU rDNA sequences from public (GenBank, TreeBASE) and private databases. We report new bioinformatic tools for identification of fungi publicly available on our website (http://iab-devel.arsc.edu/metagenomics/): 1) Similarity search with automated masking against a database of more than 28,000 fungal ITS rDNA sequences; 2) Similarity search against a database of more than 8,000 fungal LSU rDNA sequences; 3) Similarity score and sequence output of the best 50 hits in FASTA format; 4) Multiple alignment of the search result sequences. Database searching is done with an MPI (Message Passing Interface) version of FASTA across 16 cpus in a computer cluster consisting of 2 GHz Apple G5's. An MPI version of ClutstalW is used to align the top 50 hits. Automatic masking is accomplished with RepeatMasker using a custom masking library. Users enter their query sequences in a web interfaced derived from the Pise system. These tools help mycologists, fungal systematists, microbial ecologists and others to identify unknown fungi, such as species that may be recovered from the soil PCR libraries.

89. High degree of structure is detected between populations separated by short and long distances in two sympatric *Rhizopogon* species. .Lisa C. Grubisha and Thomas D. Bruns. Department of Plant and Microbial Biology, 111 Koshland, University of California, Berkeley, CA 94720-3102 USA.

We are investigating the relationship between genetic and geographic structure in two sympatric species of *Rhizopogon*. *R. vulgaris* and *R. occidentalis* are hypogeous, ectomycorrhizal fungi associated with pines. Spore dispersal is by animals that consume fruiting bodies, thus dispersal should be restricted by geographic barriers and distance between populations. *R. vulgaris* and *R. occidentalis* were sampled from native pine populations on two islands and coastal mainland sites in California. Results from analysis of microsatellite data reveal that in both species populations separated by very short distances can exhibit a high degree of genetic differentiation if intervening areas do not contain suitable habitat. Within Santa Cruz Island *R. vulgaris* populations showed a high degree of structure even though they were only separated by 11-18 km. Physical barriers, such as the lack of pines and a large dry valley, separated these populations, that were located on two mountain ranges. Very high levels of genetic differentiation between the northern California and the island populations suggest the possibility of cryptic speciation for both species. These results are consistent with the idea that in isolated pine populations gene flow between populations of these fungi is restricted by the movement of the mammals that disperse their spores.

90. Simple sequence repeat abundance and distribution in two oomycete genomes: *Phytophthora sojae* and *P. ramorum*. N. Grunwald[1], S. Tripathy[2], K. Ivors[3], K. Lamour[4] and B. Tyler[2]. [1]USDA ARS, Corvallis, OR; [2]VPI, Blacksburg, VA; [3]NC State, Fletcher, NC; [4]Univ. Tennessee, Knoxville, TN.

The first whole genome assembly of the two oomycete plant pathogens *Phytophthora sojae* (95 Mb) and *P. ramorum* (65 Mb) were examined to determine types, abundance and distribution of different simple sequence repeats (SSRs) ranging between 2 to 6 bp in motif length. We found 2,128 and 1,000 SSRs in *Ps* and *Pr*, respectively. In general, the density of SSRs (bp per Mb) in *P. sojae* is about 1.5 times that of *Pr*. Whereas AC dinucleotide repeats appear at a higher density in *Pr*, AG and AT repeats appear at a higher density in *Ps*. Interestingly, density of most trinucleotide repeats was higher in *Ps* than in *Pr*. Although *Ps* has a larger genome, the percentage of SSR loci located in coding regions is higher at 17.4% compared to *Pr* at 14.8%. Compared to other species, including *Saccharomyces cerevisiae*, repeats of length 4, 5, and 6 bp are considerably underrepresented in both *Phytophthora* genomes. Whereas in most genomes studied to date dinucleotide repeat stretches tended to be longer than other repeats, in the case of *Phytophthora* only tetranucleotide repeats were occasionally considerably longer (ACAG, ACAT, and AGAT). As expected frequency of trinucleotide repeats in exons was considerably higher when compared to di, tetra- or pentanucleotide repeats.

91. Host-associated population structure in *Septobasidium ramorum*, a fungal symbiont of scale insects. Daniel A. Henk and Rytas Vilgalys. Duke University, Durham, NC

Septobasidium ramorum is a fungal symbiont of diaspidid scale insects. The fungi form mycelial mats over entire colonies of scale insects including infected and uninfected insects. The fungus-insect relationship may be mutualistic from the perspective of a scale insect colony, but infected insects are sterile. The accepted fungal lifecycle requires frequent anastomosis of putatively recombinant genotypes within colonies and codispersal with local host scale insects. We use DNA sequence data from single spore isolates, tissue isolates, and isolates from within infected scale insects to confirm the recombinant nature of fungi infecting individual insects and detect genetic structure within fungal colonies. We also use DNA sequence data to quantify the relative host specificity and selectivity of *S. ramorum* and to assess copopulation genetic structure of *S. ramorum* and its primary host scale insect, *Chionaspis nyssae*. Results suggest that *S. ramorum* has a wide host range but its population structure largely mirrors that of its primary host insect. Comparisons to species with overlapping geographic range but different host specificity will be discussed.

92. The *Coprinopsis cinerea* laccase multi-gene family and evolution of multi-copper oxidase genes in basidiomycetes. P. Hoegger[1], S. Kilaru[1], J. Thacker[2], T. James[2], R. Vilgalys[2] and U. Kües[1]. [1]Georg-August-University Göttingen, Institute of Forest Botany, Göttingen, Germany; [2]Duke University, Department of Biology, Durham, NC.

Laccases belong to the group of multi-copper oxidases and catalyze the oxidation of phenolic compounds. We have identified a laccase multi-gene family in the white-rot *Coprinopsis cinerea* comprising 17 non-allelic laccase genes. Based on intron distributions and deduced amino acid sequences, they divide into two distinct groups. Several ancient and more recent duplication and recombination events within the two groups seem to be responsible for the observed number and genomic organisation of the genes. In phylogenetic analysis of almost 300 multi-copper proteins from insects, plants, fungi and bacteria all formed distinct clades containing all the sequences from a given kingdom and mostly according to their function (where known). In other completely sequenced genomes of basidiomycetes (*Phanerochaete, Ustilago, Cryptococcus*), we found only multi-copper oxidase genes with rather low similarities to the *C. cinerea* laccase proteins. These sequences cluster with ferroxidases, ascorbate oxidases, or enzymes of melanin synthesis. Within the fungi, the basidiomycetes appear to form a clade of typical laccases distinct from the ascomycetes. The basidiomycete sequences seem to diverge according to different life styles of the organisms, partially independent from organismal relationships.
Work in Göttingen is supported by the Deutsche Bundesstiftung Umwelt (DBU).

93. Horizontal transfer of selfing in the ascomycete genus *Stemphylium* (Pleosporales). Patrik Inderbitzin, Jennifer Harkness and Mary L. Berbee. University of British Columbia, Department of Botany, Vancouver, Canada, V6T 1Z4

The genus *Stemphylium* contains selfing species that evolved from outcrossing ancestors. To find out how selfing originated, we analyzed the *Stemphylium* MAT loci that regulate sexual reproduction in ascomycetes, and compared MAT structures and phylogeny with a multigene *Stemphylium* species phylogeny. We found that some *Stemphylium* species' MAT loci contained a single gene, either *MAT1-1* or *MAT1-2*, while others contained a unique fusion of the *MAT1-1* and *MAT1-2* regions. In all fused MAT regions, *MAT1-1* was inverted and joined to a forward oriented *MAT1-2* region. As in the closely related *Cochliobolus*, *Stemphylium* species with fused MAT regions were able to self. Structural and phylogenetic analyses of the MAT loci showed that the selfing-conferring fused MAT regions were monophyletic with strong support. However, in an organismal phylogeny of *Stemphylium* species based on 114 isolates and the four loci ITS, *GPD*, *EF-1 alpha* and *vmaA-vpsA*, selfers with identical fused MAT regions arose in two clades, each time with strong support. We showed that a one-time origin of the fused MAT loci followed by a horizontal transfer across lineages was compatible with the data. Another group of selfers in *Stemphylium* only had forward oriented *MAT1-1* at their MAT loci, constituting an additional and third origin of selfing in *Stemphylium*. We would like to acknowledge financial support from NSERC (grants to M. Berbee and P. Inderbitzin), NSF subcontract to M. Berbee (P.I. B. G. Turgeon), University of British Columbia Graduate Fellowship and a Swiss National Science Foundation Postdoctoral Fellowship to P.Inderbitzin.

94. Early diverging lineages on the fungal tree of life: phylogenetic analysis using six gene regions. Tim James[1], Joyce Longcore[2], Rytas Vilgalys[1], and the Assembling the Fungal Tree of Life (AFTOL) Working Group. [1]Dept. of Biology, Duke University, Durham, NC USA, [2]Dept. of Biol. Sciences, U. Maine, Orono, ME USA

Much of the phylogenetic and ecological diversity of the Fungi can be found in those lineages that diverged early in the history of the kingdom. These lineages include the Chytridiomycota, Zygomycota, Glomeromycota, and possibly the Microsporidia. Understanding the history of the Fungi includes unraveling the branching at the base of the kingdom, but resolution of the deepest nodes is difficult because these divergence times are ancient. The goal of the Assembling the Fungal Tree of Life project is to provide a global phylogenetic hypothesis for the Fungi using DNA sequences from multiple loci. We present preliminary results from the phylogenetic analysis of over 50 taxa of basal fungi using DNA sequences for nuclear 18S and 28S rRNA genes, ATP6, EF1-alpha, RPB1, and RPB2. There is a general lack of congruence among the phylogenies estimated from each of the individual genes. Whether the Chytridiomycetes or Zygomycetes are monophyletic remains uncertain. The rDNA data suggest the Glomeromycetes form a clade with the Ascomycota+Basidiomycota, while the data from protein encoding genes place the Glomeromycetes with the Zygomycetes. No relationship was observed between the Microsporidia and any other basal fungal lineage sampled.

95. Avirulence genes in European populations of *Leptosphaeria maculans*, the cause of stem canker of crucifers. Anna Stachowiak [1], Julia Olechnowicz [1], Malgorzata Jedryczka [1], Thierry Rouxel [2], Marie-Hélene Balesdent [2], Ingrid Happstadius [3], Peter Gladders [4], Neal Evans[5]. [1]Institute of Plant Genetics PAS, Poznan, Poland. [2]INRA, PMDV, Versailles, France. [3]Svalöf Weibull AB, Svalöv, Sweden. [4]ADAS Boxworth, Cambridge, UK. [5]Rothamsted Research, Harpenden, UK

The fungus *Leptosphaeria maculans* is the cause of stem canker (blackleg) of crucifers, the damaging disease of oilseed rape in Europe, Canada and Australia. At present, nine avirulence genes of the pathogen (*AvrLm1-AvrLm9*) are known, and these correspond to *RLm1-RLm9* resistance genes in the host. A survey was done to investigate the distribution of avirulence genes in the *L. maculans* population across Europe. The EU-funded project SECURE (StEm Canker of oilseed rape: molecular tools and mathematical modelling to deploy dUrable REsistance: QLK5-CT-2002-01813) followed a sampling protocol used during a previous large scale survey done in France. Isolates were then screened using a cotyledon test on a differential set of nine cultivars developed by INRA-Versailles. The race structure in Poland, UK, Germany and Sweden resembled the structure observed in France, with all isolates having *AvrLm6* and *AvrLm7*, but no *AvrLm2, AvrLm3* and *AvrLm9*. The distribution of *AvrLm1, AvrLm4* and *AvrLm5* ranged between countries and experiment sites, with a prevalence of a virulent allele.

96. Genetic diversity of *Ophiostoma clavigerum* associated with the mountain pine beetle, *Dendroctonus ponderosae* Hopkins in North America. S. Lee and C. Breuil. Department of Wood Science, University of British Columbia, 2424 Main Mall, Vancouver, BC, V6T 1Z4, Canada

In this work, the genetic diversity of the *Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington was assessed. This pathogenic, sapstaining fungus is associated with the mountain pine beetle (*Dendroctonus ponderosae* Hopkins), which is a native insect in North America. As of 2004, in British Columbia only, 108 million m^3 of lodgepole pine (*Pinus contorta*) has been infested by the mountain pine beetle. In 2003, *O. clavigerum* was isolated from the infested lodgepole pines in Canada (Banff in Alberta, Ft.St.James, Houston, Williams Lake, and Kamloops in British Columbia) and USA (Hidden Valley in Montana, and Hell Roaring in Idaho). A total of one hundred eighty six single-spore isolates were analyzed for their genetic variability by the amplified fragment length polymorphism (AFLP) markers generated with six primer sets. In this work, we described the genetic variation and differentiation within/among the *O. clavigerum* populations. We also examined the possible correlation of genetic distances with geographic distances among sampling locations. Overall, it appeared that the genetic polymorphism in *O. clavigerum* is low, which might be related to relatively rare sexual reproduction.

97. Progesterone decreases cAMP content in filamentous fungus *Rhizopus nigricans*. Helena Lenasi, Natasa Jeraj and Katja Breskvar. Institute of Biochemistry, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia

Mammalian steroid hormones are not present in the natural environment of a saprophytic fungus *Rhizopus nigricans*, but when cultivated *in vitro* fungal growth is selectively inhibited by progesterone, deoxycorticosterone, testosterone and estradiol. The most efficient inhibitor is progesterone. Previous studies revealed progesterone receptors (Kd = 70nM) coupled to G proteins in the plasma membrane fraction of the fungus. The biological role of progesterone binding to membrane receptors as well as the resulting signalling pathway is not known yet. In this report we examined possible signalling pathways (MAPK pathway, routes including inositol-3-phosphate and cAMP) which might follow membrane receptor activation. A remarkable decrease of cAMP content (24±14.3 to 17.1±11.0 pmol/mg protein) was observed in the presence of progesterone (>15 M). Progesterone decrease of cAMP content was not influenced by pertussis toxin or cholera toxin indicating the possibility that in *R. nigricans* Galpha subunits other than mammalian Galphas or Galphai were involved in this signalling. Progesterone also drastically changed fungal morphology. However, cAMP-mediated progesterone signalling contributes only a small part to the total growth inhibition by progesterone, therefore an additional non-specific action of progesterone should be taken into consideration.

98. Molecular evolution in the endopolygalacturonase I of *Botrytis cinerea*. C. Lévis, C. Giraud-Delville and E. Fournier. INRA-PMDV, Route de Saint-Cyr, F-78026 Versailles, France.
Identifying genes responsible for adaptive differences between populations and species may help understanding the mechanisms of evolutionary diversification. Adaptive differences should leave a signature of positive selection at the molecular level. In fungal plant pathogens, genes involved in fungus-plant interactions are candidate genes on which rapid positive selection may operate.
Endopolygalacturonases are fungal enzymes produced early during plant-pathogen interactions, e.g. the endopolygalacturonase I (BCPG1) of the Ascomycete *Botrytis cinerea* Pers. (ten Have et al. 2001, Fungal Genet Biol. 33). This species complex comprises two cryptic groups : Group II, attacking about 200 plants, and Group I with a more restricted host range (Fournier et al. 2003, Mycologia 95). In this multi-host pathogen, selection by the host may have operate. It has been shown that BCPG I less aggresive *B. cinerea* isolates BCPG I elicites a defense reaction in the plant (Poinsot et al. 2003, MPMI 16). BCPG I is therefore a good candidate gene that may be shaped by positive selection.
We tested this hypothesis by sequencing BCPG I and surrounding regions (1000 bp upstream, 2500 bp downstream) in 12 *B. cinerea* isolates (2 Group I, 10 Group II). An ORF was found in the 3' region, coding for a putative N-acetyl-transeferase. Tree topologies and evolution speeds were compared in each region. Ratio of nonsynonymous to synonymous mutation were estimated in the two coding regions. Results will be discussed in the poster.

99. Natural Variation in the Amino Terminal Polyglutamine Domain of Neurospora crassa WHITE COLLAR-1 Confers Circadian Acuity. Todd P. Michael[1], Sohyun Park[2], Tae-Sung Kim[2], Joanne Chory[1], and Kwangwon Lee[2]. [1]Plant Biology Laboratory, The Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA 92037. [2]Department of Plant Pathology, 201 Bradfield Hall, Cornell University, Ithaca, NY 14853

Natural variation in the circadian clock provides acuity for specific environments. The circadian clock synchronizes the internal biology of an organism to the exact 24-hour period of the local external environment so biological processes occur at precise times over the day despite seasonal changes. Utilizing an inverted race tube assay, which eliminates the need for the band mutation, we found that 144 *Neurospora crassa* accessions display variation in circadian parameters such as period, phase and temperature compensation. In order to understand the impact of natural variation on the underlying molecular mechanisms of the circadian clock we evaluated known clock components. We found that WHITE COLLAR-1 (WC-1), which plays dual roles as both a blue light photoreceptor and central clock component, displays variation across the 144 accessions in its amino-terminal and carboxy-terminal glutamine repeats. Utilizing F1 hybrids between accessions with different N-terminal glutamine repeats we confirm a relationship between the N-terminal glutamine repeat size and circadian period. We propose that the plastic nature of the activation domains in WC-1 may increase the fitness of an organism through optimizing the acuity of the circadian clock.

100. The impact of commercial applications of the entomopathogenic fungus *Beauveria bassiana* on the genetic diversity of indigenous conspecific populations. Prashant K. Mishra[1], Louela A. Castrillo[2], Eleanor Groden[1], John D. Vandenberg[3], and Seanna L. Annis[1]. [1]Department of Biological Sciences, University of Maine, Orono, ME04469; [2]Department of Entomology, Cornell University, Ithaca, NY14853; [3]Plant, Soil, and Nutrition Lab, USDA-ARS, Ithaca, NY14853.

The entomopathogenic fungus *Beauveria bassiana* has been widely used for the biological control of various agricultural insect pests. In this study, we have analyzed the genetic diversity in the indigenous populations of this fungus and the changes that have occurred after the application of a commercial formulation of *B. bassiana* strain GHA (Emerald BioAgriculture Corp., Lansing, MI). Amplified fragment length polymorphisms and random amplified polymorphic DNA markers were generated from *B. bassiana* isolates collected from three sprayed and three unsprayed fields. There was displacement of indigenous genotypes 1 to 2 years after application, however the frequency of indigenous genotypes increased 4 years after treatment. Isolates were assigned to four ancestral genetic populations, 1 GHA type and 3 indigenous types. Isolates from nonsprayed fields were only assigned to indigenous genetic populations. In sprayed fields, some isolates had mixed ancestry from GHA and indigenous genetic populations indicating the occurrence of recombination. This study can serve as a model for assessing the risk associated with the release of genetically modified fungi on their conspecifics.

101. Origin and migration of *Ustilago maydis*. Andrew Munkacsi[1] and Georgiana May [2]. [1]Plant Biological Sciences Graduate Program, University of Minnesota. [2]Department of Ecology, Evolution and Behavior, University of Minnesota.

Ustilago maydis is a fungus that only infects maize and the wild relatives of maize, the teosintes. The domestication and spread of maize is well understood. Maize was domesticated by humans in southern Mexico from a teosinte 6,000-10,000 years ago, and exported by humans to the United States 1,000-2,000 years ago and to South America 2,000-4,000 years ago. Unlike the evolutionary history of maize, the origin and migration of *U. maydis* has yet to be elucidated. A popular hypothesis is that the ancestral *U. maydis* population was in Mexico and *U. maydis* followed maize as it was traded to new locations. To test this hypothesis, we genotyped 900 collections of *U. maydis* teliospores from Mexico, the United States, and South America using 10 microsatellite markers. Based on this dataset, we infer the geographic location of the ancestral population, predict whether the host of that population was maize or a teosinte, and estimate the divergence times between genetically distinct populations. Together, we compare the origin and migration of *U. maydis* to the origin and migration of maize.

102. Phylogenetic Analysis of Septin Gene Family. Fangfang Pan, Russell Malmberg, Michelle Momany. Plant Biology Department, University of Georgia, 30602

Members of the septin gene family have been found in animals, fungi, and some primitive eukaryotes. They play important roles in cytokinesis and cell surface growth. To understand the evolution of the septin gene family, criteria for deciding whether a sequence may be classified as a septin have been developed. GTP-binding domains are highly conserved in septins. These motifs can be used to distinguish septins from other members of the GTPase superfamily. Some septins also have a distinctive coiled-coil domain. Approximately 170 septin and septin-like genes were identified by PSI-Blast database search for this analysis.
Phylogenetic trees were built for this family using MrBayes. There was a large diversification of septin sequences prior to the fungal/animal split. Most members of the septin gene family can be grouped with one of the S. cerevisiae septins Cdc3, Cdc10, Cdc11 or Cdc12. Seven possible new members of the septin gene family were found, including septin-like genes in more ancient organisms. Future experimental studies are needed to verify the identity of the newly found septin genes.

103. Evolution of *Ascochyta* spp. on wild and cultivated legumes. Tobin L. Peever, Marco Hernandez-Bello, Maneesha Barve & Walter J. Kaiser. Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430, USA.

Ascochyta spp. were sampled from chickpea (*Cicer arietinum*), faba bean (*Vicia faba*), lentil (*Lens culinaris*), pea (*Pisum sativum*), hairy vetch (*Vicia villosa*) and several of their wild relatives in various geographic locations worldwide. Previous results from laboratory crosses indicated that *Ascochyta* spp. infecting faba bean, lentil and chickpea should each be considered biological species. We tested the hypothesis that these biological species were also phylogenetic species and extended the phylogeny to include isolates sampled from other cultivated legumes and their related wild relatives. We sequenced the nuclear ribosomal internal transcribed spacer (ITS) and 3 protein-coding loci and estimated a phylogeny for each dataset independently and for the combined dataset. Phylogenies estimated from all genomic regions were congruent and the protein-coding loci revealed significantly more variation than ITS. The combined phylogeny revealed 6 clades, each with high bootstrap support and associated with a single cultivated host. Isolates from wild chickpea (*C. ervoides* and *C. monbretti*), wild pea (*P. elatius*) and several wild vetch species clustered with isolates from their respective cultivated hosts. Isolates from bigflower vetch (*V. grandiflora*) were polyphyletic. *Ascochyta* spp. isolated from pea and faba bean were host specific and easily crossed in the laboratory. Progeny were phenotypically normal but most were unable to infect either pea or faba bean. Phylogenetic analyses, host inoculations and in vitro genetic crosses with *Ascochyta* spp. will be useful in studying fungal speciation and the genetics of host specificity at the species level in plant-pathogenic fungi.

104. Analysis of the complete mitochondrial genomes from *Phakopsora pachyrhizi* and *Phakopsora meibomiae*. Martha L. Posada-Buitrago[1], Jeffrey L. Boore[1] and Reid D. Frederick[2]. [1]DOE Joint Genome Institute. 2800 Mitchell Drive, Walnut Creek, CA 94598. USDA-ARS Foreign Disease-Weed Science Research Unit, 1301 Ditto Avenue, Fort Detrick, MD 21702.

The complete nucleotide sequence of the mitochondrial (mt) genome was determined for two obligate fungal pathogens, *Phakopsora pachyrhizi* and *P. meibomiae*, the causing agents of the soybean rust. These 32 kb genomes contain the genes encoding ATP synthase subunits 6, 8, and 9 (atp6, atp8, and atp9), cytochrome oxidase subunits I, II, and III (cox1, cox2, and cox3), apocytochrome b (cob), reduced nicotinamide adenine dinucleotide ubiquinone oxireductase subunits (nad1, nad2, nad3, nad4, nad4L, nad5, and nad6), the large and small mitochondrial ribosomal RNAs and tRNAs for all amino acids. Gene order and gene sequences were compared with all available complete fungal mtDNAs-representatives of all four Phyla.

105. Fungal Inteins and Horizontal Gene Transfer. Russell Poulter, Margi Butler, Jeremy Gray and Tim Goodwin. Department of Biochemistry, University of Otago, Dunedin New Zealand

There are only two described nuclear encoded inteins. One is the VMA intein (which includes a homing endonuclease domain) found in some hemiascomycetous yeasts. We have described a second intein in the basidiomycete yeast *Cryptococcus neoformans*, a fungal pathogen of humans. This intein is encoded as an in-frame insertion within the nuclear PRP8 gene. The intein includes the domains associated with intein splicing, but lacks the homing endonuclease domain associated with intein mobility. This intein is present in all *C. neoformans* strains examined and in all strains of the closely related pathogenic species, *Cryptococcus bacillisporus*. The intein was not found in most related *Cryptococcus* species or in other basidiomycetes. PRP8 inteins were found in several ascomycetes including *Histoplasma capsulatum, Aspergillus nidulans, Aspergillus fumigatus*, and its close relative *Neosartorya fischeri*. These inteins are at the identical site in the PRP8 sequence. PRP8 inteins were not found in other ascomycetes. Unlike the *Cryptococcus neoformans* and *C. bacillisporus* inteins, some of the ascomycete PRP8 inteins contain homing endonuclease domains and are thus potentially mobile. Comparisons of the shared splicing domains reveal that the ascomycete and cryptococcal inteins are surprisingly similar in sequence, given the time since these fungi diverged from a common ancestor (~550 Mya). We propose that the discontinuous phylogenetic distribution of the intein is the result of a horizontal gene transfer.

106. Transposition, Recombination and Gene Genesis in *Magnaporthe grisea.* Powell AJ, Pan H, Diener SE and Dean RA. Fungal Genomics Laboratory North Carolina State University

Magnaporthe grisea (f. sp. *oryzae*) is the premier model organism for study of fungal pathogenic interactions with cereal crops. Recent advances in this model include acquisition of the genome sequence, large-scale mutational analyses and global transcriptional studies using oligo-based microarrays. The latter global transcriptional studies revealed the apparent upregulated expression of LTR retrotransposon *maggy* in asexual spores (conidia), relative to mycelia and appressoria. RT PCR analyses of *gag* and *pol*, constituent ORFs of *maggy*, corroborated our microarray findings. Two formal possibilities could account for the observed pattern of expression: either *gag* and *pol* ORFs were autonomously transposing or these ORFs had inserted into upregulated resident genes, and were thus "hitchhikers," and not truly actively transposing. Rigorous bioinformatic analyses revealed the presence of known and predicted genes containing different partial copies of maggy. These partial copies represent the full spectrum of *maggy* structural features, from *gag* and *pol* ORFs, to LTR regions. The *maggy*-derived genes are therefore chimeric ORFs, where recipient genes have acquired modules of non-homologous sequence, from which evolution can shape novel forms and functions. Preliminary RT PCR and EST data indicate that *maggy*-derived chimeric ORFs are expressed. Our results are significant because they represent the discovery of a novel class of chimeric genes that show developmentally distinct patterns of expression. These chimeras are likely the product of transpositional recombination, an expected source of genomic novelty. Detailed structural, functional and evolutionary characterization of these *maggy*-derived chimeras, as well as those arising from other transposable elements, promises to broaden our understanding of genomic, organismal and population levels of biological complexity.

107. Withdrawn

108. Assessing reproductive mode in the mitotic entomopathogen, Beauveria bassiana using gene genealogies, allelic associations and distribution of mating-type idiomorphs. Stephen A. Rehner and Ellen P. Buckley. USDA-ARS, Insect Biocontrol Laboratory, Beltsville, MD, USA.

The entomopathogenic fungus, Beauveria bassiana , is renowned for its copious production of mitotic, haploid conidia and the conspicuous absence of a sexual stage throughout most of its global range. To determine reproductive mode in this species, an essential first step is the circumscription of appropriate population samples in which the contrasting reproductive alternatives of clonality and recombination can be critically investigated. We outline our implementation of the phylogenetic species recognition (PSR) criterion to identify regionally sympatric groups of related individuals for population-level analyses. We focus on two closely related terminal clades: one from eastern Asia, which includes a mix of sexual and asexual isolates, and a second from western Europe, in which only asexual reproduction has been observed. Statistical tests of allele associations among polymorphic microsatellite loci indicate that each population is recombining. The structural organization of the mating-type locus is described, demonstrating that *B. bassiana* possesses a heterothallic mating-type system with canonical MAT1 and MAT2 specificities. Evidence is presented that the MAT1 and MAT2 mating-types are randomly distributed in the European population, supporting the conclusion that genetic recombination arises through sexual reproduction.

109. Populations, clones and individual mycelia of arbuscular mycorrhizal fungi. Søren Rosendahl and Eva H Stukenbrock, Department of Microbiology, Institute of Biology, University of Copenhagen, Denmark

Population genetic analyses of arbuscular mycorrhizal fungi (AMF) are often hampered by the obligate biotrophy of the fungi. Multiple markers from single spores can be obtained by nested multiplex PCR method using a combination of primers for simultaneous amplification of five loci in one PCR reaction. This approach was used for multilocus genotyping of arbuscular mycorrhizal fungal populations sampled hierarchically from an organically and a conventionally cultured field. All spore genotypes were unique suggesting that no recombination was taking place in the populations. There were no overall differences in the distribution of genotypes in the two fields and identical genotypes could be sampled from both fields. Analysis of gene diversity showed that Glomus populations are subdivided between plots within each field, while there was no subdivision between fields. This suggests that individuals related to the dominant mycelial network are favoured while the establishment of genetically different individuals is restricted. This hypothesis is supported by studies of AMF in natural vegetation where the community is dominated by even larger mycelial networks that may interconnect different plant species.

110. Mating system transitions in the euascomycete genus *Aspergillus* subgenus *Fumigati* section *Fumigati.* Carla Rydholm[1], Mathieu Paoletti[2], Paul S. Dyer[2], Francois Lutzoni[1]. [1] Duke University, North Carolina. [2] University of Nottingham, Nottingham, UK.

The evolutionary origin of *Aspergillus fumigatus* is of applied and theoretical interest. This study has uncovered data which suggests the divergence of this species was coincident with a mating system transition from homothallism to heterothallism. To test this hypothesis, a combined approach is used where the arrangements of mating loci and their flanking genes are characterized for multiple heterothallic and homothallic species from *Aspergillus* subgenus *Fumigati* section *Fumigati*. The likelihood of heterothallism as the derived state for *A. fumigatus* and other heterothallics of the group is estimated within a phylogenetic framework using ancestral character state reconstruction techniques. At this point, preliminary results for both types of evidence, from the organization of the mating loci and reconstruction of ancestral state, support a transition from homothallism to heterothallism having taken place within the genus *Aspergillus* subgenus *Fumigati* section *Fumigati.*

111. Population genetics of *Aspergillus fumigatus* and its close relative *Neosartorya fischeri*. Carla Rydholm[1], George Szakacs[2], Francois Lutzoni[1]. [1] Duke University, North Carolina, USA. [2] Technical University of Budapest, Budapest, Hungary.

Aspergillus fumigatus is a saprophytic euascomycete mold with a ubiquitous presence worldwide. It is also a pervasive pathogen to immunosuppressed patients. Despite intensive work to understand its success as a pathogen, little information is known regarding the population dynamics and recent evolutionary history of this species. We examined patterns of variation at three intergenic loci in a sample of 60 natural isolates from various parts of the world. These loci were also used to analyze site specific variation for 30 strains isolated from five localities. For both data sets no evidence of population structure was detected and there was no association between the genetic and geographic distances among different natural isolates. Thus, nothwithstanding its nearly worldwide occurrence, little evidence was found for local adaptation in *A. fumigatus* strains derived from diverse locations. Since conflicting observations have been made as to the amount of genetic variation within *A. fumigatus*, we contrast its effective population size with that of its closest relative, *Neosartorya fischeri*, and conclude *A. fumigatus* has comparatively low intraspecific genetic variation. Based on linkage disequilibrium measures *A. fumigatus* is inferred to have a recombining population structure.

112. Naturally Occurring Hybrids of *Fusarium graminearum*. Amgad A. Saleh, John F. Leslie, and Robert L. Bowden. Department of Plant Pathology, Kansas State University, Manhattan, KS

Fusarium graminearum (*Gibberella zeae*) is a major cause of cereal scab, in particular Fusarium scab (head blight) of wheat. DNA sequence analyses have been used to define nine phylogenetic lineages that have been accorded species rank. In this study, we used DNA sequence from four nuclear genes (*MAT-1-13*, *alpha-tubulin*, *reductase*, and *Tri101*) and AFLP analyses to determine if hybrids between these lineages are present under field conditions. We analyzed 200 isolates of *F. graminearum* collected from wheat and sorghum at eleven locations in Brazil and Uruguay. Three fields contained isolates of more than one lineage. In these fields, lineage 7 strains represented more than 90% of the sample. We found hybrids between lineages 1 and 7; 2 and 7; and 7 and 8. Our data suggest that there are no substantial barriers to gene flow between different lineages in the same location, and that the different phylogenetic lineages belong to a single biological species.

113. A Phylogenomic Approach to Reconstructing the Diversification of Proteases in Fungi. G. Hu and R.J. St. Leger. University of Maryland, Department of Entomology, College Park, MD 20742

Gene duplication and loss are important mechanistic antecedents in generating functional diversification. Here we adopted a phylogenomic approach with fungi of very different virulence and habitat to survey and characterize their serine proteinases (subtilases and trypsins) with the goal of providing a framework of information on these important enzymes, as well as improving understanding of general processes in fungal gene family evolution. The survey was based on 9 fungal genomes and expressed sequence tags from the insect pathogen *Metarhizium anisopliae*. Comparing subtilases between species revealed that basidiomycetes (*Cryptococcus neoformans*, *Coprinus cinereus*, *Ustilago maydis*) and saprophytic ascomycetes (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Aspergillus nidulans*, *Neurospora crassa*) lack the large gene families encoding secreted enzymes found in the pathogenic ascomycetes (*M. anisopliae*, *Magnaporthe grisea*, *Fusarium graminearum*). Patterns of intron loss and the degree of divergence between paralogs indicate that the proliferation of subtilisins classes I and II in pathogens occurred after the basidiomycete/ascomycete split but predated radiation of ascomycete lineages. This suggests that the early ascomycetes had a lifestyle that selected for multiple proteases, while the current disparity in gene numbers between ascomycete lineages results from retention of genes in pathogens that have been lost in saprophytes. However, the pathogens retained and occasionally expanded different gene families. Thus, *M. grisea* has 15 class I subtilisins and 6 class II subtilisins, while *M. anisopliae* and *F. graminearum* each possess 11 class II subtilisins but three or fewer class I subtilisins. This reveals evolutionary selection of different gene families among Ascomycete fungi. A prevailing trend towards lineage specific gene loss was shown by the distribution of trypsins across 35 representative fungi. Trypsin genes are lacking in most saprophytes, but are present in a basidiomycete insect symbiont (*Septobasidium canescens*), most zygomycetes and many ascomycete plant and insect pathogens. The patchy distribution of trypsins suggest that their phylogenetic breadth will have been much wider in early fungi than currently and confirms the dynamic nature of the fungal genome.

114. Molecular Adaptations for Host Range Diversification in a Fungal Pathogen. G. Hu, F.M. Freimoser and R.J. St. Leger. University of Maryland, Department of Entomology, College Park, MD 20742

Host pathogen interactions are an important force shaping organismal diversity, yet little is known about the evolution of genes responsible for virulence in pathogens. The tremendous amount of genetic variation, distinct disease phenotypes and host ranges of strains of the insect pathogen *Metarhizium anisopliae* have made it an excellent model to study the role of gene duplication/divergence in generating the functional diversification of enzymes and toxins necessary for adaptation to different hosts. To illustrate this, we present examples where strains with broad host ranges and strains with very narrow host ranges have diverged through changes in gene regulation, gene duplication/loss, and gene divergence.

115. Fungal Intron Evolution. Jason E Stajich[1], Scott W Roy[2], Fred S Dietrich[1]. [1]Department of Molecular Genetics and Microbiology Duke University, Durham, NC. [2] Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA

Understanding the evolutionary changes in exon and intron structure is an important aspect of gene evolution. The recent genome sequencing projects have given us a rich sampling of fungal genomes to study the changes in gene structure. The availability of the sequence for over 25 fungal genomes including intron rich genomes of Basidiomycete and Euascomycete fungi and the intron poor Hemiascomycetes permit the investigation of intron evolution in the fungal kingdom.
We have evaluated alignments of orthologous sequences to identify putatively orthologous introns, those which fell with in or between the same codons in the alignment of the genes. This analysis allowed us to observe the number of shared intron positions among the different fungal genes and identify many introns which are putatively ancient among the Euascomycete and Basidiomycete clades.
We used a maximum likelihood framework to reconstruct the ancestral density of introns in 800 orthologous genes across the Basidiomycota, Hemiascomycota, and Euascomycota and found evidence of massive and recurrent episodes of intron loss. This analysis suggests while intron gain may play some role, intron loss is the dominant force in fungal gene structure evolution.

116. Delineation of species boundaries within Fusarium graminearum, the causative agent of Fusarium Head Blight. D. E. Starkey, USDA, NCAUR, MGB, Peoria, IL, 61604; T. J. Ward, USDA, NCAUR, MGB, Peoria, IL, 61604; K. L. O'Donnell, USDA, NCAUR, MGB, Peoria, IL, 61604; D. M. Geiser, Dept. of Plant Pathology, Pennsylvania State Univ., University Park, PA 16802; G. Kuldau, Dept. of Plant Pathology, Pennsylvania State Univ., University Park, PA 16802; R. M. Clear, Canadian Grain Commission, Winnipeg, MB R3C 3G8; L. R. Gale, USDA Cereal Disease Lab, St. Paul, MN 55108; H. C. Kistler, USDA Cereal Disease Lab, St. Paul, MN 55108, and T. Aoki, MAFF, Tsukuba, Japan.

The primary etiological agent of Fusarium Head Blight (FHB), Fusarium graminearum, has been regarded as a single panmictic species. However, phylogenetic analyses of multilocus DNA sequence data has indicated that this morphospecies comprises at least 9 phylogenetically distinct, and biogeographically structured species (hereafter referred to as the Fg clade) using genealogical concordance phylogenetic species recognition (O'Donnell et al., 2000; Taylor et al., 2000; Ward et al., 2002). In a preliminary screening of FHB strains, O'Donnell et al. (2004) formally proposed species rank for the eight unnamed cryptic species within the Fg clade. In this study, a panel of 2520 FHB strains from around the world was screened to determine the species' geographic and host distribution, using portions of two phylogenetically informative genes (EF1-alpha and reductase, 1601 bp/strain). The trichothecene toxin chemotype of each strain was also determined using a multiplex PCR assay targeting TRI3 and TRI12 (Ward et al. 2002). The present study resulted in the discovery of 4 cryptic species, including a novel clade in Australia and the presence of 6 Fg clade species within the U.S. The development and application of robust molecular tools, such as those described herein, for Fg clade species identification and chemotype determination will significantly improve disease surveillance and global monitoring efforts, and will make available for the first time detailed information on the geographic and host distributions of FHB pathogens and enhance the current knowledge of the ecology, epidemiology and population dynamics of these mycotoxigenic cereal pathogens.

117. Development and amplification of multiple co-dominant genetic markers from single spores of arbuscular mycorrhizal fungi by nested multiplex PCR. Eva H. Stukenbrock and Søren Rosendahl. Department of Microbiology, Institute of Biology, University of Copenhagen, Oester Farimagsgade 2D, DK-1353 Copenhagen K, Denmark

Multilocus genotyping of arbuscular mycorrhizal fungi has so far only been carried out using dominant markers as AFLP and ISSR. The interpretation of these dominant markers may however be difficult as homology of the polymorphic loci cannot be ensured. Here we report the development of a nested multiplex PCR method which allows amplification of multiple co-dominant markers from single spores of Glomus mosseae, G. caledonium and G. geosporum in one PCR reaction.
The polymorphic co-dominant markers were identified and characterized in intron regions in the three protein coding genes GmFOX2, GmTOR2 and GmGIN1 by sequencing and SSCP. Additionally, we included Glomus specific primers for the well characterized LSU rDNA D2 region in the nested multiplex PCR. Seven different isolates of G. mosseae were characterized as seven unique multilocus genotypes. We found only one sequence type of each protein coding gene in each spore suggesting a homokaryotic structure of the multikaryotic Glomus spores.
Multilocus genotyping of Glomus single spores using co-dominant markers provide an important tool for future studies of evolution and population genetics of these obligate biotrophic fungi.

118. Development of a PCR-RFLP-based identification system for *Fusarium asiaticum* and genetic characterization of western Japanese isolates. H. Suga[1], G.W. Karugia[2], T. Ward[3], L. R. Gale[4], K. Tomimura[5], T. Nakajima[5], K. Kageyama[6], M. Hyakumachi[2]
[1]Life Science Research Center, Gifu University, Japan; [2]Fac. of Applied Biological Sciences, Gifu University, Japan; [3]National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL, USA; [4]USDA-ARS Cereal Disease Laboratory, St. Paul, MN, USA; [5]KONARC, National Agriculture and Bio-oriented Research Organization, Japan; [6]River Basin Research Center, Gifu University, Japan

The *Fusarium graminearum* clade consists of at least nine phylogenetically and sometimes biogeographically distinct species. Two Japanese isolates have previously been shown to belong to *Fusarium asiaticum* O'Donnell, T. Aoki, Kistler et Geiser (=*Fusarium graminearum* lineage 6). To further investigate the Japanese population, 175 isolates were obtained from wheat and barley with head blight symptoms collected from western Japan in 2002. All 18 randomly selected isolates had *F. asiaticum*-unique nucleotides in their reductase gene although two main sequence types were found among them. One of the unique nucleotides was present in a *Mse*I recognition site of both main types and this restriction site was used for the development of a PCR-RFLP-based identification system for *Fusarium asiaticum* and characterization of all isolates. Additionally, isolates were subjected to PCR-based trichothecene chemotyping (15 acetyl deoxynivalenol: 15ADON, 3 acetyl deoxynivalenol : 3ADON or nivalenol: NIV). Among 170 isolates that were identified as *F. asiaticum*, 107 (63%), 62 (36%), and 1(1%) were of the NIV, 3ADON and 15ADON type, respectively. Reductase gene sequencing identified three isolates as *F. graminearum* O'Donnell, T. Aoki, Kistler et Geiser (=*Fusarium graminearum* lineage 7). Among these, two isolates were of the 3ADON and one of the 15ADON type. Using 10 VNTR markers, all 26 randomly selected *F. asiaticum* isolates displayed different haplotypes; therefore a high level of genetic diversity was observed.

119. Development of Simple Sequence Repeat Markers for *Puccinia graminis* and *P. triticina*. Les J. Szabo and Kim Phuong Nguyen, USDA ARS Cereal Disease Lab, University of Minnesota, St. Paul, Minnesota, USA

Rusts are obligate plant parasitic fungi with a complex life cycle that often include five different spore stages. As a result, molecular studies are often performed with the asexual, dikaryotic uredinial stage (urediniospores) using dominant markers (RAPDs, AFLPs). Currently, molecular genetic and population genetic studies are underway with the wheat rust fungi *Puccinia graminis* f.sp. *tritici* and *P. triticina* the casual agents of wheat stem rust and wheat leaf rust, respectively. The lack of co-dominant molecular markers for genetic analysis has been a significant impediment. Simple sequence repeat (SSR) enriched libraries were constructed from *P. graminis* and *P. triticina* genomic DNA. Approximately, 200 clones from each library were screened and sequenced. Sixty SSR markers have been developed from the *P. graminis* clones and the majority consists of di-nucleotide (AG and GT) repeats ranging from 10 to 28 repeats in length. Eleven of the repeats were complex and often contained degenerate motifs. Thirty of the markers have been screened against ten *P. graminis* North American isolates, 27 of which were polymorphic and detected from 2 to 10 alleles per marker. Fifty-six markers have been developed from the *P. triticina* clones, the majority of which consists of di-nucleotide (AG and GT) repeats and 13 consist of tri-nucleotide repeats (CAA). Screening of these markers against a select set of *P. triticina* isolates have begun.

120. Unusual insertions of phylogenetic relevance in the nuclear ITS region of some Peronosporaceae (Oomycetes). Marco Thines[1], Hedwig Komjati[2], Mark Bachofer[1], Otmar Spring[1]. [1]Institute of Botany of the University of Hohenheim, Stuttgart, Germany, [2]Department of Plant Protection of the Szent Istvan University, Goedoelloe, Hungary

The ITS region is a sequence routinely screened for the elucidation of the phylogeny of related species. For the Peronosporales, some of which cause severe economic loss in agriculture, no such sequence data are available for a broad array of taxa. Amplification of the nuclear ITS region in *Plasmopara halstedii* resulted in a 2700bp fragment, which could not be sequenced by primer walking, due to repeated elements in the ITS-2 part. A restriction-ligation technique uncovered four tandemly arranged elements of 322bp in length each. These are part of an insertion found neither in *Phytophthora* nor in *Peronospora* species, in which total ITS-lengths of about 900bp were observed. The elements revealed variable parts in comparison to each other and were divided by short spacers of 34bp to 38bp in length. The tandemly arranged elements showed sequence variation in samples of different geographic origin, in contrast to the uniformity of the other parts of the ITS. Other species of *Plasmopara* and *Bremia* revealed lengths of ITS up to 3kB. These lengths are also derived from similar insertions, which may become a tool to trace the phylogeny of the Peronosporaceae. A comparative analysis of the repetitions in *Plasmopara halstedii* and *Bremia lactucae* will be presented.

121. Population genetics of the aflatoxigenic species, *Aspergillus flavus* and *Aspergillus parasiticus*. Nai Tran-Dinh[1], John Pitt[1] and Dee Carter[2]. [1]Food Science Australia, CSIRO, North Ryde, NSW, Australia. [2]School of Molecular and Microbial Biosciences, The University of Sydney, Sydney, NSW, Australia.

Aspergillus flavus and *Aspergillus parasiticus* are closely related, morphologically similar species belonging to the *Aspergillus* section *Flavi*. Both species can produce aflatoxins, but not all strains of either species do so. Aflatoxin contamination of crops such as peanuts and corn is a worldwide problem. A possible solution is the use of nontoxigenic strains of *Aspergillus* as biocontrol agents to competitively exclude their toxigenic counterparts in the field. Knowledge of genetic diversity, strain identification and the potential for genetic exchange are essential for predicting the likely success of such a strategy. RAPD and microsatellite analysis of 35 Australia strains distinguished *A. flavus* from *A. parasiticus* strains. *A. flavus* strains were further divided into two distinct groups; Groups 1 and 2. Group 1 and 2 *A. flavus* strains differed in their toxin production profiles, and mode of reproduction analysis revealed evidence for recombination in Group 1 *A. flavus* but not in Group 2. These differences suggested that Group 2 *A. flavus* are a new species. A global survey of 296 strains, including strains from Africa, Asia, Oceania, Europe, North and South America, using microsatellites, revealed 271 different genotypes and that both *A. flavus* and *A. parasiticus* strains showed no correlation between genotype and geographic origin or ability to produce aflatoxin. The majority of *A. flavus* strains studied clustered within Group 1 *A. flavus*. Group 2 *A. flavus* were mainly restricted to the Southern Hemisphere. Implications for a biocontrol strategy are discussed.

122. Molecular markers used for speciation in the genus *Alternaria*. Nai Tran-Dinh, Ailsa Hocking and John Pitt. Food Science Australia, CSIRO, North Ryde, NSW, Australia.

The genus *Alternaria* is a major problem in Australian grains (wheat, sorghum, barley) and is also involved in post-harvest loss of citrus fruit and tomatoes. Some *Alternaria* species are toxigenic, producing a range of toxins including tenuazonic acid, which has been shown to be toxic to plants, animals and insects. Despite its importance, speciation of *Alternaria* infecting crops is poorly understood and delineated. *Alternaria* species are principally identified by microscopic examination of conidial morphology and occasionally by some biochemical properties. Identification of *Alternaria* species by such methods is often difficult and requires specialised training. Conidial morphologies of various species have overlapping features, and some morphological characters are not consistently expressed in culture. Environmental conditions also affect the variability of morphological characteristics. With over 1000 *Alternaria* species names having been published since the genus was named in 1817, some confusion exists in its taxonomy. The advent of molecular techniques and their application to fungal taxonomy has provided an additional tool for speciation. This study uses various molecular markers for the identification of *Alternaria* species including amplified fragment length polymorphisms and microsatellite markers. A collection of type strains, including the *Alternaria* species *A. alternata*, *A. citri*, *A. infectoria*, *A. solani*, and *A. tomato*, were used to access the suitability of these molecular markers for the identification of *Alternaria* species.

123. Application of three molecular marker techniques to characterise strains of *Aspergillus niger*. Alexandre Esteban[1,2], Su-lin L. Leong[1,3,4] and Nai Tran-Dinh[1]. [1]Food Science Australia, PO Box 52, North Ryde NSW 1670, Australia. [2]Departament de Sanitat i d'Anatomia Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, E-08193 Bellaterra, Barcelona, Spain. [3]School of Agriculture and Wine, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia. [4]Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, SA 5064, Australia

Aspergillus niger is a member of the black aspergilli (*Aspergillus* sect. *Nigri*) and is a common food spoilage fungus. *A. niger* holds GRAS (Generally Regarded As Safe) status and is widely used in the food industry as a source of hydrolytic enzymes and organic acids. However, some strains of *A. niger* are able to produce the nephrotoxin, ochratoxin A (OTA), and have been associated with OTA in coffee and grape products. Despite its importance, the taxonomy of *A. niger*, and other members of the black aspergilli, remains unclear. Species identification is primarily based on morphological criteria, but a growing number of molecular techniques are being applied to the *A. niger* aggregate. RFLP analyses have divided the *A. niger* aggregate into types N and T, and RAPD analyses have shown a high level of intraspecific variability. The molecular techniques, Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR, Amplified Fragment Length Polymorphisms (AFLP) and microsatellite markers were evaluated for their suitability in typing strains of *A. niger*. Strains assessed included isolates from culture collections and those isolated from natural substrates including grapes, coffee, animal feed and soil. The collection included both toxigenic and nontoxigenic isolates. ERIC-PCR differentiated *A. niger* from other closely related black aspergilli. AFLP analysis separated *A. niger* strains into types N and T, and this was confirmed by analysis using six novel microsatellite markers, developed for *A. niger*. Furthermore, both the AFLP and microsatellite analyses separated type N strains into two distinct groups. No correlation was seen between toxin production and genotype.

124. Multi-gene phylogeny and evolution of thraustochytrids. Clement K.M. Tsui, W. Marshall, D. Honda*, R. Yokoyama*, M.L. Berbee. Department of Botany, #3529-6270 University Blvd., The University of British Columbia, Vancouver, B.C., Canada V6T 1Z4 *Department of Biology, Faculty of Science and Engineering, Konan University, Kobe

The labyrinthulomycetes are fungal-like protists composed of thraustochytrids, aplanochytrids and labyrinthulids. The thraustochytrids, including three major genera – Thraustochytrium, Schizochytrium, and Ulkenia, are abundant heterotrophs in marine and mangroves habitats. Previous phylogenetic investigation based on SSU rDNA revealed that none of the genera Thraustochytrium, Schizochytrium and Ulkenia is monophyletic, indicating that the morphological characters are unreliable as taxonomic criteria. So we are applying multi protein-coding gene phylogenies (Elongation factor 1-alpha, beta-tubulin, actin) to re-evaluate the relationships among these genera and species. Preliminary results from analysis of individual gene and combined data appear to be congruent to rDNA that these genera do not form monophyletic groups. We will also attempt to evaluate the phylogenetic relationships among different groups of stramenopiles that include non-photosynthetic and photosynthetic protists whose zoospore flagella bear tripartite tubular hairs. Additional strains are also being isolated from marine animals, as part of the investigation of eukaryotic diversity. Their SSU rDNA sequences are determined and included in the phylogenetic analysis. Results indicated that there are at least eight distinct taxa within labyrinthulomycetes, and their protein genes will be further investigated and analysed.

125. Partitioning reproductive isolation in *Neurospora:* measuring the strength of component reproductive isolation barriers and their relative contributions to isolation between species. Elizabeth Turner, David J. Jacobson, John W. Taylor. University of California, Berkeley, USA

Sexual isolation between closely related species results from the cumulative effects of several isolating barriers acting at various points throughout the reproductive pathway. To understand how reproductive isolation evolves and how genetic isolation is maintained, it is necessary to understand the nature of the barriers, the strength of the barriers, and the contribution of each barrier to total reproductive isolation. Experiments that dissect the effects of individual barriers allow us to estimate what proportion of heterospecific gene flow a given barrier prevents and to assess natural variation in barrier strength and the effects that different mating conditions have on barrier performance. The contribution of a given barrier depends both on the strength of that barrier and the strengths of all barriers that precede it in the sexual pathway, and barriers acting earlier in the pathway will have a disproportionately large effect. We demonstrate the utility of this method by analyzing experiments that partition reproductive isolation between *Neurospora crassa* and *N. intermedia* into the following components: trichogyne/conidium interactions, perithecia production, spore melanization, spore viability, and viable spore production. We find that barrier strength varies between pairs of strains and that reproductive isolation profile is altered when females are simultaneously fertilized by conspecific and heterospecific males.

126. Reinforcement of reproductive isolation barriers in *Neurospora:* selective advantage and genetic basis of the early abortion of hybrid perithecia. Elizabeth Turner, David J. Jacobson, John W. Taylor. University of California, Berkeley, USA.

Reproductive isolation barriers between lineages can evolve by several different mechanisms. Both neutral and selected changes will accumulate in diverging genomes and these can render hybrids between lineages inviable or unfit due to negative epistasis. In a process termed "reinforcement," isolation barriers can themselves be positively selected if they prevent the wasteful investment of reproductive effort in the formation of unfit hybrids. The signature of reinforcement is reproductive isolation barriers that are stronger in sympatry versus allopatry. In particular, barriers will be positively selected that permit females to discriminate between hybrid and conspecific matings. Previous work from this lab has shown that hybrid matings between *Neurospora* individuals from sympatric populations do experience stronger barriers than matings between allopatric individuals. We now present results of two sets of experiments exploring a potential fitness advantage and the genetic basis of one putative reinforcement barrier, the very early abortion of *N. crassa* peritheicia fertilized by sympatric males of *N. intermedia*. First we show that this barrier can dramatically increase the overall fecundity of these females if they have additional opportunities to mate with conspecific males. Second we identify a major quantitative trait locus associated with the reinforcement phenotype.

127. Improved gene replacement in *Aspergillus awamori* by *Agrobacterium*-mediated transformation. C.B. Michielse[1], M. Arentshorst[1], A.F.J. Ram[1,2] and C.A.M.J.J. van den Hondel[1,2]. [1]Leiden University, Institute of Biology Leiden, Fungal Genetics Research Group, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands, [2]Department of Applied Microbiology and Gene Technology, TNO-Nutrition, 3700 AJ Zeist, The Netherlands.
The efficiency of gene replacement in *A. awamori* between *Agrobacterium*-mediated transformation and CaCl2/PEG mediated transformation has been compared. For two genes targeted, it was found that the homologous recombination frequencies obtained by *Agrobacterium*-mediated transformation were 3 to 6-fold higher than the frequencies obtained with the CaCl2/PEG mediated transformation method. Furthermore, it was found that efficient homologous recombination with shorter DNA flanks could be obtained in *Agrobacterium*-mediated transformation. Finally, the addition of the dominant *amdS* marker as a second selection marker to the gene replacement cassette led to a further 2-fold enrichment of putative gene replacement transformants, resulting in a homologous recombination frequency of 55%. Based on the data it can be concluded that *Agrobacterium*-mediated transformation is an efficient tool for gene replacement and that the *amdS* gene can be successfully used as a second selection marker to enrich the pool of putative gene replacement transformants

128. Generation of a large set of microsatellite-markers for *Phytophthora infestans* by mining sequence data. Theo van der Lee[1], Odette Mendes[1], Hannneke van der Schoot[1], Caroline Ruyter-Spira[1], Bas te Lintel Hekkert[1], Francine Govers[2] and Gert H. J. Kema[1]. Plant Sciences Group, [1]Plant Research International and [2]Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands

Microsatellite or simple-sequence repeat (SSR) markers are extremely useful in population studies particularly in diploid species. So far, however, only a limited number of informative microsatellite loci have been identified in the potato late blight pathogen *Phytophthora infestans* and none have been mapped. To identify additional polymorphic SSR loci, genomic and EST sequences were scanned for the presence of di- and trinucleotide units that are repeated six or more times. We identified 333 unique SSR loci using an automated software pipeline. Primers flanking these SSRs were developed and tested on a set of ten previously characterized *P. infestans* field isolates. Of the 300 primers pairs tested, 203 pairs generated a clear fragment of the expected length. More than half of these amplified microsatellites (110) showed length differences among the different isolates. Some microsatellites seemed to be very variable in the *P. infestans* population with up to 9 different alleles detected in the ten genotypes. The most informative microsatellites are currently being positioned on the genetic linkage map of cross 71 (80029 x 88133). The genomic and EST sequences (xgi.ncgr.org/spc/; Randall et al., 2005 MPMI in press) proved to be an excellent source for new microsatellites. We anticipate that these SSR markers will be instrumental for efficient analyses of *P. infestans* populations and will facilitate integration of the various genetic datasets available for this important plant pathogen.

129. Phylogeography of the *Ustilago maydis* Virus in the USA and Mexico. Voth, Peter[1], Lockhart, Ben E.[2] and May, Georgiana[1]. Depts. of Plant Biological Sciences[1] and Plant Pathology[2], University of Minnesota, St. Paul, MN USA

Symbiotic interactions strongly affect the evolutionary trajectory of the participating symbionts and are important in structuring communities. These interactions are such that the population genetic structure of one symbiont can affect that of the other. One such symbiosis is that of *Ustilago maydis, Ustilago maydis* Virus (UMV), and *Zea mays*. *Ustilago maydis*, commonly known as corn smut, causes infections on vegetative and reproductive tissues of corn. UMV is vertically transmitted through cytoplasmic fusion during mating of *U. maydis* individuals and, thus, the population dynamics of *U. maydis* can affect the population structure of UMV. In this work, I investigate the evolutionary interaction of a dsRNA virus (UMV) with *U. maydis* to determine the phylogeography of UMV throughout the USA and Mexico. I have collected sequence data from regions of two genes on the UMV genome and assessed infection frequency, phylogenetics, genetic diversity, pairwise Fst, and recombination frequencies. These analyses have revealed that Mexican populations of UMV demonstrate higher genetic diversity and infection rates than do populations from the USA, local populations in the USA are composed of multiple UMV lineages, and populations of UMV are not in equilibrium with mutation, migration, and drift.

130. A Multilocus SNP Microsphere Array for Identification of Fusarium Head Blight Species and Chemotypes. Todd J. Ward, David Starkey, Brent Page, and Kerry O'Donnell. Microbial Genomics & Bioprocessing Research Unit, National Center for Agricultural Utilization Research, USDA Agricultural Research Service, Peoria, IL 61604

Fusarium head blight (FHB) poses a serious constraint to the production of wheat and barley worldwide. Infested grains are often unsuitable for food or feed due to contamination with trichothecene toxins and estrogenic compounds. In addition, FHB generally causes a significant reduction in seed quality and yields. A detailed understanding of pathogen diversity is crucial for effective disease control programs that minimize the threat of FHB. It appears that only a fraction of FHB species/chemotype diversity is currently represented within North America. Therefore, the introduction of novel FHB pathogens or chemotypes via global trade in agricultural products has the potential to exacerbate the FHB problem in the U.S. Using a unique multi-locus DNA sequence database (11 nuclear genes, 13.6 kb of DNA sequence) we have developed a high-throughput single tube assay for the simultaneous identification of all known B-trichothecene FHB species and chemotypes in order to improve disease surveillance efforts and to facilitate a greater understanding of the ecology, epidemiology, and population dynamics of these FHB pathogens.

131. Vegetative compatibility groups in *Botryotinia fuckeliana*. Pauline L Weeds, and Ross E Beever. Landcare Research, Auckland, New Zealand.

A vegetative compatibility system is known to occur in the plant pathogenic fungus *Botryotinia fuckeliana* (*Botrytis cinerea*). We have used complementing nitrate-non utilising (Nit) mutants to study the distribution and inheritance of vegetative compatibility groups (VCGs). Field populations were found to comprise many VCGs, with 60 identified amongst 71 field strains. While most VCGs contained a single strain, 5 contained 2 or more members. A sample of 28 single ascospore progeny from a sexual cross between 2 field strains in different VCGs generated 9 VCGs, including both parental types. Considering both field and single ascospore strains we now recognise 67 VCGs for *B. fuckeliana*. Our findings are consistent with the presence of multiple vegetative compatibility (*vic*) genes; if 2 alleles are assumed for each gene, our data suggest the existence of at least 7 such genes. Genetic diversity of the field populations was assessed using 8 microsatellite markers. Some field strains (25%) showed 2 alleles for one or more markers, consistent with the hypothesis they are heterokaryons. Excluding these strains, most field strains had different haplotypes, except for those collected in a hierachical manner in one vineyard block, where there was evidence of clonality and inbreeding. The parents of the sexual cross differed for 5 of the microsatellite markers. These segregated approximately 1:1 and none were closely linked. A total of 14 haplotypes were detected amongst the 28 progeny, although neither of the parental haplotypes was recovered.

132. Examination of the phylogeny and possible function of genes specific to *A. fumigatus* and *A. fischerianus*. Jennifer R. Wortman[1], Natalie D. Fedorova[1], Charles Lu[1], Jonathan Badger[1], Michael J. Anderson[2], Neil Hall[1], Jonathan A. Eisen[1], William C. Nierman[1]. [1]The Institute for Genomic Research, Rockville, MD, USA. [2]The University of Manchester, Manchester, UK.

Comparative analysis of the genomes of *Aspergillus fumigatus*, a human pathogen, with the model organism *Aspergillus nidulans* and the industrial agent *Aspergillus oryzae* has led to the identification of more than 500 genes that appear specific to *A. fumigatus*. Provocatively, many of these *A. fumigatus* genes have few or no homologs in the genomes of other eukaryotes available in public databases. Instead, a subset shows significant sequence similarity to bacterial or archaeal proteins. Interesting examples include heat shock protein GroES, death-on-curing family protein Doc, and arsenate reductase ArsC.
The preliminary genomic sequence and automated annotation of a fourth Aspergillus genome, *Aspergillus fischerianus* (*Neosartorya fischeri*), has allowed us to investigate further the *A. fumigatus*-specific gene set. As *A. fischerianus* is the most closely related species to *A. fumigatus* that has been identified (apart from the sister species *A fumigatus var. ellipticus*), we can now determine that approximately half of the genes previously identified as *A. fumigatus*-specific genes are actually shared between *A. fumigatus* and *A. fischerianus*. We are also investigating genes that appear unique to *A. fischerianus*.
Some of these *A. fumigatus* and *A. fischerianus*-specific genes are likely to confer significant selective advantage to these fungi in competing for resources and in adapting to diverse environments. We are currently exploring the origin of these genes using phylogenetic methods in order to posit whether they were selectively retained by these species or acquired through lateral gene transfer.

133. Biodiversity in Ten members of Stachybotrys from Ukraine through molecular analysis of the small subunit ribosomal DNA. Veronica Amaku, Oliver Rojas, Gary Andersen, Tamas Torok, Duane Pierson, Mark Ott, and Olufisayo Jejelowo

Members of the genus *Stachybotrys* are of interest due to the implication of *Stachybotrys chartarum* in sick building syndrome and infant pulmonary hemosiderosis. Current identification techniques which involve cultural and microscopical identification of colonies, conidia and hyphae, are time consuming and subjective. They depend on human evaluation, which varies according to the level of the analyst's expertise and experience. Recent advances in molecular biology provide tools that can be exploited to improve accuracy and time required for fungal identification. Although the use of molecular techniques for fungal identification is very promising, it is in infant stage and still being explored. In this study, we analyzed the small subunit ribosomal DNA of ten members of the genus *Stachybotrys* using PCR and Sanger sequencing. Four of the isolates matched similar sequences in existing nucleotide databases, three isolates could not be amplified, and sequences from the remaining three isolates matched *Penicillium species*.

134. Sequencing and annotation of the genome of the human pathogenic basidiomycete *Cryptococcus neoformans* serotype D, Strain JEC21. Paolo Amedeo, Paola Roncaglia, Iain Anderson, Rama Maiti, Bernard B. Suh, Brian J. Haas, Jonathan E. Allen, Mihaela Pertea, Jessica Vamathevan, Viktoriya Grinberg, Florenta R. Riggs, Terry R. Utterback, Steven L. Salzberg, Jennifer R. Wortman, Brendan J. Loftus, Claire M. Fraser

Cryptococcus neoformans is a soil borne basidiomycete growing as encapsulated yeast and responsible of serious respiratory diseases and meningitis in humans. Endemic of Australia, Vancouver Island (since recently) and many other places having mild climate, it is frequently isolated ubiquitously from soil and, more copiously, from birds' excrements.
It's clinical importance has risen during the last decades due to AIDS epidemic and the usage of immunosuppressive drugs to treat autoimmune diseases and after organ transplantations.
Five main serotypes (A, B, C, D, and A-D) of *C. neoformans sensu lato* are known, being serotype A the most important from a clinical perspective, but serotype D the first of which strains of opposite mating types were isolated allowing genetic studies.
Strain JEC21 has been completely sequenced and fully assembled in chromosomes virtually without any sequence gap. Gene structure have been manually curated and alternatively spliced isoforms have been identified thanks the alignment of the end-sequences of about 23000 full-length cDNAs.
In this poster we describe the sequencing and annotation of this strain and we compare it with the sequence draft of a partially co-genic strain (B3501A) sequenced by Stanford Genome Technology Center.

135. Viewing Annotated Aspergillus Genomes on the Central Aspergillus Data Repository (Cadre) Website. JEM Gilsenan MJ Anderson TK Attwood SG Oliver NW Paton GD Robson DW Denning. The University of Manchester, Manchester, UK.

CADRE has been funded to house publicly available genomic data for all Aspergillus species. The Ensembl database schema and software have been adapted and used to set up a Website (http://www.cadre.man.ac.uk/). Using a Web browser, the user can view the position of mapped features, such as protein-coding genes, on sequence contigs. For each feature, links are provided to allow the user to retrieve further data. For protein-coding genes, such data include: chromosomal co-ordinates; a description of the encoded protein's function; similarity matches; and displays of transcript structure and protein features. Two complete Aspergillus genomes are currently available on the Website. The A. nidulans genomic sequence and annotation has been provided by the Broad Institute and consists of 28.6Mb of DNA with 9520 genes. The sequence consists of 248 contigs assembled into 27 supercontigs. The next release of the A. nidulans sequence will incorporate manually corrected gene reports brought to our attention during the writing of the genome sequence paper. The A. fumigatus sequence was determined by The Institute for Genomic Research (TIGR) and the Sanger Institute. Annotation was generated automatically and manual changes have been incorporated by TIGR during the process of writing the genome paper. The genome consists of 28.8Mb of DNA with 10,034 genes. The sequence has been assembled into 16 chromosomal arms stretching from the telomere to the centromere. Two arms contain gaps: one of these is only 5kb and the other one represents the ribosomal DNA repeat.

136. The *Candida* Genome Database (CGD), a curated community resource for *Candida albicans* gene and protein information. Martha B. Arnaud, Maria C. Costanzo, Marek S. Skrzypek, Gail Binkley, Christopher Lane, Stuart R. Miyasato, and Gavin Sherlock. Stanford University School of Medicine, Stanford, CA

The *Candida* Genome Database (CGD) contains genomic information and provides community resources for researchers who are interested in the opportunistic fungal pathogen *Candida albicans*. CGD is freely available at http://www.candidagenome.org/. CGD displays *C. albicans* information collected from the scientific literature, including *C. albicans* gene names and aliases; Gene Ontology (GO) terms describing the molecular function, biological process, and subcellular localization of each gene product; mutant phenotypes; and free-text description lines to summarize the function and biological context of each gene product. CGD also provides community resources. At the request of the community, CGD has implemented a gene name reservation system to facilitate gene name assignment before publication and to avoid future publication of conflicting gene names. CGD also hosts a colleague registry through which *Candida* researchers can share contact information and research interests. CGD is currently under development, and we welcome comments and suggestions. To contact CGD curators, send email to candida-curator@genome.stanford.edu. CGD is supported by NIH grant R01 DE15873-01 from the NIDCR at the NIH.

137. The *Neurospora crassa* community genome annotation project. Heather M. Hood[1], James E. Galagan[2], Bruce W. Birren[2], Jay C. Dunlap[3], & Matthew S. Sachs[1]. [1]Oregon Health & Science University, Beaverton, OR 97006; [2]Broad Institute, Cambridge, MA 02141; [3]Dartmouth Medical School, Hanover, NH 03755.

The *Neurospora crassa* genome sequence was released in 2001. Automated annotation predicted ~10,000 genes. While automated annotation, including prediction of protein-coding regions and intron-exon boundaries, is crucial, errors in automated processes occur and they have limited ability to assign functional properties to genes. To produce richer and more accurate annotation, manual annotation and curation is necessary. Our goals are to improve annotation by manual curation and to integrate phenotypic annotation with genomic sequence. Substantial phenotypic information already exists for approximately 1000 loci and ongoing targeted gene-knockout experiments are expanding phenotypic knowledge of genes identified directly from sequence data. To produce the most valuable annotation data are establishing a controlled vocabulary to describe phenotypes associated with mutations in specific genes. At this conference, we are releasing a prototype web-based community annotation resource. This will allow community experts to submit data for associating mutant allele phenotypic information with specific genes and for improving gene-structure models. Curators will assess submissions and integrate these data into the genome annotation. This project will vastly improve the utility of the *Neurospora* genome by providing manual and curated community annotation, and by integrating phenotypic data with sequence data.

138. The Podospora Genome Project. Philippe Silar[1], Patrick Wincker[2], Fabienne Malagnac[1], Eric Espagne[1], Antoine Boivin[3], Olivier Lespinet[1], Amid Khalili[1], Robert Debuchy[1], Sylvie Arnaise[1], Veronique Berteaux-Lecellier[1], Corinne Clave[4], Veronique Contamine[1], Evelyne Coppin[1], Arnaud Couloux[2], Corinne Dasilva[2], Fons Debets[5], Michelle Dequard-Chablat[1], Rolf Hoekstra[5], Marguerite Picard[1], Berangere Pinan-Lucarre[4], Annie Sainsart-Chanet[3], Sven Saupe[4], Carole H. Sellem[3] and Jean Weissenbach[2]. [1] Institut de Genetique et Microbiologie, UMR8621, Orsay, France. [2] Genoscope, Evry, France. [3] Centre de Genetique Moleculaire, UPR2167, Gif sur Yvette, France. [4] Institut de Biochimie et Genetique Cellulaires, UMR5095, Bordeaux, France. [5] Laboratory of Genetics, Wageningen University, Wageningen, Netherland.

Using a whole genome shotgun approach, we sequenced the *Podospora anserina* genome to a 10X coverage. Arachne assembly led to 2911 contigs totaling 35 Mbp, close to pulsed-field gel estimate of genome size. The N50 consists of 232 contigs greater than 42 kb and 18 supercontigs greater than 550 kbp. Previously identified genetic markers and 150 newly discovered microsatellites allowed 53 supercontigs to be anchored to the genetic map. ESTs obtained from various stages of Podospora life cycle are currently sequenced. Data are posted at http://podospora.igmors.u-psud.fr .

139. Microarray analysis of vegetative incompatibility in *Neurospora crassa.* Sarah C. Brown, Takao Kasuga, Isao Kaneko and N. Louise Glass. University of California, Berkeley

In *Neurospora crassa*, hyphal fusion between genetically distinct individuals leads to the formation of heterokaryons. If hyphal fusion occurs between individuals with different alleles at any het locus, growth arrest, hyphal compartmentation and cell death occur. We are investigating this vegetative incompatibility using a temperature sensitive mutant capable of forming stable heterokaryons at 34˚C with an otherwise incompatible partner. After transfer to 20˚C the incompatible phenotype is evident. We are using oligonucleotide arrays to compare gene expression patterns in compatible and incompatible heterokaryons made with the temperature sensitive mutant at 34˚C and 20˚C. Preliminary results indicate the involvement of a number of known cell rescue genes and a high proportion of predicted genes of unknown function.
A microarray analysis of heterokaryons undergoing vegetative incompatibility will provide us with a global picture of changes in gene expression, giving a clearer understanding of the regulation of transcription during vegetative incompatibility in *Neurospora crassa*. We aim to identify genes that play an active role in the control of cell death during vegetative incompatibility.

140. A proteomic approach to identify extracellular and cell wall proteins involved in the *Phytophthora infestans* – plant interaction. Catherine R Bruce, Shuang Li, Neil AR Gow and Pieter van West. The Aberdeen Oomycete Group, College of Life Sciences and Medicine, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, Scotland, UK.

A thorough understanding of the molecular events taking place during interactions between *Phytophthora infestans* and host and non-host plants is crucial for developing new control strategies. At the plant-pathogen interface, an exchange of molecular signals is thought to determine the outcome of the interaction. We anticipate that secreted and cell wall proteins will be rich in important signalling molecules involved in disease resistance or establishing successful infection. A proteomic approach is employed to identify novel extracellular and cell wall proteins from mycelia cultured in vitro and from plant intercellular fluid during *P. infestans* – tomato interaction. At present we have identified over 40 protein spots. Several of these may represent effector molecules and these are characterised further. Here we present our latest results.

141. Molecular Dissection of *Neotyphodium lolii* / Perennial Ryegrass Symbiosis. Richard Johnson, Christine Voisey, Shalome Bassett, Susanne Rasmussen, Linda Johnson, Charlotte Gaborit and Gregory Bryan. AgResearch Ltd., Tennent Drive, Private Bag 11008, Palmerston North, New Zealand.

Neotyphodium lolii is a fungal endophyte that lives entirely within the intercellular spaces of its grass host, perennial ryegrass. Infection is symptomless and the endophyte relies on the host plant for dissemination via the seed. The association is mutually beneficial since the endophyte confers a number of biotic and abiotic advantages to the host, including enhanced plant growth, protection from certain mammalian and insect herbivores, enhanced resistance to nematodes and some fungal diseases.
We are using a multidisciplinary approach to dissect the molecular basis of this symbiosis and intend to link the knowledge gained from basic biology and cytology with various functional genomics approaches. We have used suppressive subtractive hybridisation, fungal EST and targeted genome sequencing to develop a sequence database containing over 5000 fungal genes. The ESTs and other genes have been used to develop a microarray (see L. Johnson et al.). We are using proteomics to correlate fungal proteins expressed in culture with those expressed in planta including secreted proteins which may play a role in signalling between the host and fungus. By linking these approaches we hope to identify genes which are important in both the establishment and maintenance of symbiosis. We are interested in signalling between the fungus and its host and have isolated several signalling genes including adenylate cyclase and a MAP kinase for targeted gene disruption studies. We have also isolated several secondary metabolite biosynthesis gene clusters and are currently investigating their role in symbiosis (see R. Johnson et al. and D. Fleetwood et al.).

142. The role of *SPO11* in meiosis and meiotic recombination in *Neurospora crassa.* F.J. Bowring, P.J. Yeadon, R.G. Stainer and D.E.A. Catcheside. School of Biological Sciences, Flinders University, PO Box 2100, Adelaide SA 5001, Australia.

A homologue of the yeast *SPO11* gene is carried by numerous organisms, including humans, and is thought to code for the enzyme that catalyses the initiation of all meiotic recombination. We have generated three RIP mutant alleles and several deletion strains of the Neurospora crassa *SPO11* homologue.
When homozygous, all three RIP alleles reduce fertility and spore viability, and an analysis of the remaining viable spores suggests extensive chromosome non-disjunction during meiosis. Microscopic analysis of mutant perithecial tissue reveals a spectrum of meiotic defects including a failure of homologous chromosomes to synapse during pachynema. While we expected that, as in other organisms, mutation of the *SPO11* homologue would reduce or abolish recombination in Neurospora, our data indicate this is not the case. The frequency of allelic and non-allelic recombination near the recombination hotspot cog^{L} is at least preserved and possibly elevated in mutant crosses although recombination in another region of the genome may be suppressed. Preliminary analysis of meiosis in strains having the *SPO11* homologue deleted suggests that the three RIP mutants harbour null alleles.

143. Use of yeast recombinational cloning and a *Neurospora crassa* strain defective in non-homologous end joining for high-throughput production of gene replacement mutants. Hildur V. Colot, Gyungsoon Park*, Carol Ringelberg, Susan Curilla, Christopher Crew*, Jennifer J. Loros, Katherine A. Borkovich* and Jay C. Dunlap. Genetics Department, Dartmouth Medical School, Hanover, NH; and *Department of Plant Pathology, University of California, Riverside, CA

We will be creating knockout (KO) mutants for all annotated *Neurospora* genes as part of an NIH-funded Program Project (PO1). We previously reported development of a strategy suitable for high-throughput gene deletions, involving the creation of *hph*-marked KO cassettes by recombination-mediated plasmid construction in *S. cerevisiae* (Asilomar 2004), followed by transformation of split-marker fragments into *Neurospora*. We have adapted our yeast recombinational cloning techniques for use on a Beckman Biomek NX robot and have now created KO cassettes for thousands of *Neurospora* genes. We have also improved our protocol for creating the final *Neurospora* KO mutants by utilizing a *Neurospora* mutant defective in nonhomologous end-joining (NHEJ) as the recipient for electroporation. Recent work from the Hirokazu Inoue laboratory (Ninomiya et. al., 2004) demonstrated that mutation of genes involved in NHEJ repair (*mus-51*/ku70 and *mus-52*/ku80; both genes replaced with *hph*) leads to a high rate of homologous recombination in *Neurospora*. We have subsequently created our own *mus-51* and *mus-52* KO strains using the selectable marker *bar*. We have confirmed that electroporation of either of these mutants with several KO constructs containing 1 kb of flanking DNA on either side of the *hph* marker results in 100% of the transformants possessing the correct gene replacement and no ectopic insertions of any portion of the cassette. Generation of homokaryotic KO mutants for an initial group of 100 transcription factor genes is in progress, and data on their characterization will be presented.

144. *Saccharomyces* Genome Database: a resource for fungal comparative genomics. Maria C. Costanzo[1], Rama Balakrishnan[1], Karen R. Christie[1], Kara Dolinski[2], Selina S. Dwight[1], Stacia R. Engel[1], Dianna G. Fisk[1], Jodi E. Hirschman[1], Eurie L. Hong[1], Robert Nash[1], Rose Oughtred[2], Anand Sethuraman[1], Marek S. Skrzypek[1], Chandra L. Theesfeld[1], Gail Binkley[1], Qing Dong[1], Stuart Miyasato[1], Mayank Thanawala[1], Shuai Weng[1], David Botstein[2], J. Michael Cherry[1]. (1) Dept. of Genetics, Stanford University, 300 Pasteur Drive, Stanford, CA, 94305-5120, USA; (2) Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA.

The *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) is a comprehensive compilation of genome, gene, and protein information for the model fungus *S. cerevisiae*. SGD offers tools for comparison and analysis of information on a genome-wide scale, both within *S. cerevisiae* and between *S. cerevisiae* and other species. The PSI-BLAST pages present a precomputed, periodically updated PSI-BLAST analysis of each *S. cerevisiae* protein vs. the non-redundant protein set in GenBank, with results organized by taxonomic grouping. The Model Organism BLASTP Best Hits pages display links to curated database pages of proteins in other model organisms that are similar to *S. cerevisiae* proteins. The Fungal BLAST tool allows comparison of any input sequence against multiple fungal genomes. This open-source tool is based on the BLAST module developed by the GMOD project (www.gmod.org) and is readily adaptable to other databases. All information at SGD is freely available, both via the web interface and in downloadable files. SGD is supported by a grant from the National Human Genome Research Institute, NIH.

145. The Genus Alternaria as a Model for Necrotrophic Fungal Pathogens of Plants. K. D. Craven (1), R. Cramer (2), D. Knudson (3), C. B. Lawrence (4), T. K. Mitchell (1). (1) North Carolina State University, Fungal Genomics Laboratory, Raleigh, NC; (2) Duke University, Durham, NC; (3) Colorado State University, Fort Collins, CO; (4) Virginia Bioinformatics Institute, Blacksburg, VA.

We have created numerous resources to investigate the genome structure and mechanisms of pathogenicity and gene flow among plant pathogenic species in the fungal genus Alternaria. To study gene expression during compatible (disease-causing) interactions, we have generated and analyzed cDNAs from EST libraries created from Alternaria brassicicola-infected tissues of two related Brassicaceous hosts, and from fungal mycelium grown under nitrogen-starvation conditions. From a set of approximately 2000 unique fungal EST sequences, we have selected candidates for gene knockout to evaluate their effects on pathogenicity. A BAC library of the Alternaria brassicicola genome was created and is being used to elucidate genome structure, identify clusters of functionally related genes, and to contribute to the recently funded genome sequencing effort. Many distinct pathotypes of Alternaria alternata have been shown to produce host-selective toxins required for disease, and further, that the toxin biosynthetic genes are typically located on small, conditionally dispensable (CD) chromosomes. Despite their crucial role in disease development, such chromosomes are not characteristic of entire fungal species, and thus are likely to be under-represented in genome sequences of organisms containing them. We chose the tomato pathotype, A. alternata fsp. lycopersici, from which to isolate a known 1.0 Mb CD chromosome for library construction and sequencing. It has been postulated that the clustering of pathogenicity genes on CD chromosomes may allow for efficient horizontal transfer between fungal strains or species through hyphal anastomosis. We are investigating the potential for such gene flow through the construction of nitrate-utilization mutants of A. brassicicola and related Alternaria species.

146. Fungal comparative genomics – an update on the Fungal Genome Initiative. Li-Jun Ma, Christina Cuomo, Sarah Calvo, Dave DeCaprio, Jonathan Butler, Manfred Grabherr, David B. Jaffe, Tim Elkins, Shawn Samuel, Miriam Averbuch, Chinappa Kodira, Eric Lander, Chad Nusbaum, James Galagan, and Bruce Birren. The Broad/MIT, Cambridge, USA

The Fungal Genome Initiative (FGI) is generating genomic resources for organisms across the fungal kingdom by producing genome sequence, annotation and analysis. The FGI species represent the major branches of the fungal tree and will help elucidate the molecular basis for the tremendous diversity that arose in the 1 billion years of fungal evolution. In addition, we are sequencing clusters of phylogenetically closely related species to examine recent evolutionary events. These clusters include fungi that are human and plant pathogens, as well as important model systems. So far, a total of 26 fungal genome sequence projects have been completed or are in the queue. The data can be accessed at the Broad website (http://www.broad.mit.edu/annotation/fungi/fgi/). We will present an update on our sequencing progress and describe recent results.

147. Sequencing and analysis of the *Fusarium graminearum* genome. Christina Cuomo, Li-Jun Ma, Jonathan Butler, Sarah Calvo, Dave DeCaprio, Tim Elkins, James Galagan, Jin Rong Xu‡, Frances Trail†, Corby Kistler*, and Bruce Birren. Broad Institute of MIT and Harvard, Cambridge MA. *Univ. of Minnesota, St Paul MN. †Michigan State Univ., East Lansing, MI. ‡Purdue Univ., West Lafayette IN.

Fusarium graminearum is a major plant pathogen that causes head blight of wheat and barley. The Broad Institute and members of the *Fusarium* community have produced and analyzed a high-quality draft sequence of *F. graminearum*. The assembly contains 36 Mb of sequence at 10-fold depth. Nearly all (99.5%) of the sequence was anchored to the 4 chromosomes using genetic mapping. A total of 11,640 protein coding genes were predicted. 17% of proteins are unique to *F. graminearum*, showing no significant homology to any protein in the nonredundant (nr) protein database. Compared to other sequenced eukaryotes, *F. graminearum* contains very few high identity paralogous genes. Additionally, the genome is repeat poor, containing between 15 and 30-fold fewer repeats than other sequenced ascomycetes. One possible explanation for the lack of high identity sequences is that the process of repeat-induced point mutation (RIP) is active in *F. graminearum*. The longest repeat family, a group of Fot1-like inactive transposons, shows a mutation bias consistent with a low level of RIP. Further, a search of the protein set revealed a putative ortholog of the rid (rip-defective) gene of *N. crassa*. However, the genome contains very few remnants of repetitive elements as compared to *N. crassa*. This suggests that while there is some evidence for RIP activity in *F. graminearum*, RIP alone does not explain the absence of repeats in the genome. (This work was supported by the NSF/USDA, Microbial Genome Sequencing Project, award number 2002-35600-12782)

148. Genomic-directed development of new therapies to treat *Pneumocystis* infections. Melanie T. Cushion, Margaret Collins, Sandeep Bansil, Sandy Rebholz, Alan Ashbaugh, Peter D. Walzer. University of Cincinnati College of Medicine and the VAMC, 231 Albert Sabin Way, Cincinnati, OH 45267-0560

Species of the fungal genus *Pneumocystis* cause a lethal pneumonia (PcP) in mammalian hosts with compromised immune status. *Pneumocystis* lack detectable ergosterol rendering them refractory to standard anti-fungals. Increasing evidence indicates that *P. jirovecii* (the species infecting humans) is developing resistance to the most efficacious treatment, TMP-SMZ (targeting folate synthesis), and to second-line drugs (e.g. atovaquone). We re-constructed a sterol biosynthetic pathway for *P. carinii* with genomic data from the *Pneumocystis* Genome Project (http://pgp.cchmc.org). Use of specific inhibitors showed that enzymes in this pathway were functional. The effects of tolnaftate, tebuconazole, and simvastatin on gene regulation were evaluated using macroarrays. Simvastatin had little effect on sterol genes, although organism viability was decreased by 80%. Tebuconazole down-regulated its target, 14-alpha-demethylase, and other genes in the pathway. Tolnaftate down-regulated almost all sterol genes. Isobolograms showed synergy between simvastatin/tebuconazole. Simvastatin was effective in reducing lung burden in the mouse model of PcP ($p=0.0258$) vs untreated controls. Combination therapies targeting pathways other than folate synthesis hold promise for new treatment modalities for PcP while analyses of gene expression can suggest alternative drug targets (NIH-AI-50450, NO1 AI75319).

149. Phylogenomic Analyses of Heterokaryon Incompatibility Proteins in Aspergilli. Natalie D. Fedorova[1], Jennifer R Wortman[1], William C. Nierman[1]. [1]The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850, USA.

Heterokaryon incompatibility is a form of programmed cell death in filamentous fungi, triggered by the fusion of filaments from two strains with different sets of het loci. The analyses of the completely sequenced genomes of Aspergillus fumigatus, Aspergillus fischerianus, Aspergillus nidulans and Aspergillus oryzae reveal remarkable diversity of heterokaryon incompatibility systems in aspergilli and other filamentous fungi. The Aspergillus genomes encode numerous homologs of heterokaryon incompatibility proteins from Neurospora crassa and Podospora anserina. Yet the number of homologous proteins, their domain architecture and, perhaps, the underlying mechanisms are quite different.

Notable examples include an aspergilli-specific expansion of the putative NACHT NTPases that function as signaling hubs during heterokaryon incompatibility in filamentous fungi, pathogen resistance in plants, or apoptosis in mammals. The Aspergillus NACHT proteins are most similar to GTP-binding protein Het-E and Het-D from P. anserina, but they do not have the HET domain, found at the N-terminus of the Podospora proteins. Instead many of Aspergillus NACHT proteins have a highly divergent nucleoside phosphorylase domain. Still unknown is whether all NATCH NTPases are involved in self/non-self recognition during heterokaryon incompatibility or some are part of another signaling pathway in aspergilli.

150. Demonstration of LOH by SNP microarray analysis and alterations in strain morphology in strains of *Candida albicans* during infection. Anja Forche, Paul T. Magee, and Georgiana May. University of Minnesota, Saint Paul MN

Candida albicans is a diploid yeast with a predominantly clonal mode of reproduction, and no complete sexual cycle is known. As a commensal organism, it inhabits a variety of niches in humans. It becomes an opportunistic pathogen in immunocompromised patients, and can cause both superficial and disseminated infections. It has been demonstrated that genome rearrangment and genetic variation in isolates of *C. albicans* is quite common. One possible mechanism for generating genome-level variation among individuals of this primarily clonal fungus is mutation and mitotic recombination leading to loss of heterozygosity (LOH). Taking advantage of a recently published genome-wide SNP map, a SNP microarray was developed for 23 SNP loci residing on chromosomes 5, 6, and 7. It was used to examine 21 strains previously shown to have undergone mitotic recombination at the *GAL1* locus on chromosome 1 during infection in mice. In addition, karyotypes and morphological properties of these strains were evaluated. Our results show that during *in vivo* passaging, LOH events occur at observable frequencies and that such mitotic recombination events occur independently in different loci across the genome and that changes in karyotypes and alterations of phenotypic characteristics can be observed either alone, in combination, or together with LOH.

151. A genetic map of *Gibberella zeae* using sequence-tagged sites and AFLPs. Liane R. Gale[1], Je'Nise D. Bryant[2], Henriette Giese[3], Talma Katan[4], Kerry O'Donnell[5], Haruisha Suga[6], Thomas R. Usgaard[5], Todd J. Ward[5], and H. Corby Kistler[1]. [1]CDL, USDA St. Paul, MN. [2]University of Minnesota, St. Paul, MN. [3]Royal Veterinary and Agricultural University, Copenhagen, Denmark. [4]Volcani Center, Bet Dagan, Israel. [5]NCAUR, USDA, Peoria, IL. [6]Gifu University, Gifu, Japan.

A genetic map of *Gibberella zeae* (anamorph *Fusarium graminearum*) was constructed using a cross between nitrate-nonutilizing (*nit*) mutants of strain PH-1 and a Minnesota field strain, 00-676. A total of 111 ascospore progeny were analyzed for segregation at 237 loci. Genetic markers consisted of SNPs (detected as dCAPs, n=86 or CAPs, n=47), AFLPs (n=71), VNTRs (n=27), and six others. While 213 markers exhibited Mendelian inheritance, segregation distortion was noted for 17 markers at four genomic locations. A linkage map was generated using JoinMap 3.0 and a LOD threshold value of 4.0. Eleven linkage groups were obtained, covering 1154 cM and anchoring 99.8% of the sequence assembly. All linkage groups and anchored supercontigs were assembled into four chromosomes, leaving only 11 smaller supercontigs (76,055 bp total) of the nuclear DNA not anchored. Comparison between physical and genetic distance along chromosomes revealed genomic regions with reduced recombination and recombinational hotspots. More information can be found at http://www.broad.mit.edu/annotation/fungi/fusarium.

152. DelsGate a robust and rapid deletion plasmid construction method. María D. Garcia-Pedrajas and Scott E. Gold. Department of Plant Pathology, University of Georgia, Athens

A combination of PCR and gateway technology together with use of the I-*Sce*I homing endonuclease provides a rapid robust universal method for construction of plasmids suitable for precise genomic deletions in two days. We employed this method for the deletion of several genes in the basidiomycete fungus *Ustilago maydis* which causes corn smut disease. This approach, however, is universal for fungi. The method involves 2 PCR steps followed by gateway cloning to generate the final product. The first PCR reaction employs specific primers (1 and 2) 1kbp 5' and 3' of the start and stop of the ORF. The 5' end of primer 1 includes the 18bp recognition sequence of I-*Sce*I generally absent in fungal genomes. The 2nd reaction employs inverse PCR of the self-ligated 1st PCR product. Primers 3 and 4 are directed out from the ORF at the start and stop codons. These primers have at their 5' ends the *att*B1 and *att*B2 recognition sites. The 2nd PCR product, having 1kb gene flanks but lacking the entire ORF, is then inserted with B/P clonase into a donor vector with an appropriate selectable marker. After digestion with I-*Sce*I the plasmid is transformed into the fungus of interest by standard methods.

153. Comparative Genomic Hybridization within the Genus Neurospora. Luz B. Gilbert, Takao Kasuga, Jeff Townsend, Louise Glass, and John W. Taylor. U.C. Berkeley, Plant and Microbial Biology.

Comparative Genomic Hybridizations (CGH) are becoming a popular way to determine similarity among strains and even species. A growing trend is to use CGH data to assess evolutionary history by developing phylogenetic trees from differences in hybridization between isolates. As yet few have questioned the reliability of CGH data to correctly assess sequence differences in hybridization and therefore the ability of this type of data to determine evolutionary relationships. The study of a simple eukaryote, the filamentous fungus *Neurospora*, offers a unique opportunity to rigorously address these questions using both experimental and simulated data. The genus *Neurospora* consists of eight closely related conidiating species indistinguishable by morphology, as well as several non-conidiating species. An accurate phylogeny was published by Dettman et al. 2003. We have constructed a 70mer oligomer array for *Neurospora crassa* representing 10,000 genes. I have analyzed comparative genomic hybridizations for all eight conidiating species of *Neurospora* as well as a few non-conidiating isolates. These results were then compared to simulated data generated to mimic the design of the CGH experiment. The goal of the simulated data is to determine under what scenarios CGH data might accurately determine evolutionary relatedness. We have used both the simulated and empirical data to generate distance based dendograms for the different species that we can compare to the known phylogeny and assess the utility of CGH data for testing evolutionary relationships.

154. Identification from an EST database and genetic mapping of microsatellites in *Mycosphaerella graminicola*. Stephen B. Goodwin[1], Jessica R. Cavaletto[1], Theo van der Lee[2], Bas te Lintel Hekkert[2], Gert H. J. Kema[2]. [1] USDA-ARS, Crop Protection and Pest Control Research Unit, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, USA; [2] Plant Research International B.V., Wageningen, The Netherlands

Mycosphaerella graminicola, the cause of septoria tritici blotch of wheat, is developing rapidly as a genetic model for fungi in the order Dothideales. Further development of the genetics of this organism would be facilitated by the availability of microsatellite or simple-sequence repeat (SSR) markers. However, only nine microsatellite loci have been identified so far in *M. graminicola*, and none has been mapped. To identify additional polymorphic SSR loci, an EST database from *M. graminicola* was scanned for di- and trinucleotide units repeated tandemly six or more times. Among more than 30,000 EST sequences screened, 109 possible SSR loci were identified using an automated software pipeline. Primers flanking 99 of these SSRs were developed and tested for amplification and polymorphism on the two Dutch parents of the standard *M. graminicola* mapping population, one isolate from North Africa, and two from North America. Seventy-seven of the 99 primer pairs generated an easily scored banding pattern and 51 were polymorphic among the five field isolates of *M. graminicola* tested. Among these 51 loci, 23 were polymorphic between the parents of the mapping population; 21 of these plus two previously published microsatellites were integrated into the existing genetic map. These 23 microsatellite loci mapped to 12 of the 22 linkage groups of the *M. graminicola* genetic linkage map. Most (66%) of the primer pairs also amplified bands in the closely related barley pathogen *Septoria passerinii*, but only six were polymorphic among four isolates of that species tested. The EST database provided an excellent source of new microsatellites, some of which also may be useful in closely related species. These markers are highly polymorphic (up to 4 alleles per locus among five isolates tested) and can be multiplexed to facilitate integration of the different types of genetic analysis performed on this important plant pathogen.

155. Unfolded Protein Response in Aspergillus niger chemostat fermentations. T. Goosen[1,2], T. R. Jørgensen[3], J.J.L. Iversen[3], C.A.M.J.J. van den Hondel[1,2]. [1]Leiden University, Institute of Biology Leiden, Fungal Genetics Research Group, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands, [2]Department of Microbiology, TNO-Nutrition and Food Research, P.O. Box 360, 3700 AJ Zeist, The Netherlands, [3]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, 5230 Odense, Denmark

Unfolded Protein Response (UPR) is a universal reaction of eukaryotic cells to protein folding stress in the endoplasmic reticulum (ER). The expression of heterologous secreted proteins often results in such stress and thus UPR, also in filamentous fungi like Aspergillus niger which are renowned for their high secretion capacity. As a consequence, the production yield of secreted heterologous proteins usually is very low (milligrams/L at best).
UPR results in induction of expression of genes that allow the cell to cope with the surplus of protein folding intermediates. Identification of these genes and understanding the response pathway(s) should give leads to improve the folding and secretion capacity of A. niger. Towards this, two approaches are taken: transcriptomics with Affymetrix genome arrays to identify up- or down-regulated genes and genetic screening to select regulatory mutants.
For transcriptomics analysis, A. niger strains are constructed in which poorly folded (scFv) or unfoldable (yeast CPY*) proteins can be expressed under control of the regulated glaA promoter. RNA analysis is first performed with shake flask cultures, both under induced and non-induced conditions for the expression of the poorly folded proteins
For a sound transcriptomics analysis, tight control of all cultivation conditions is an absolute prerequisite. We therefore are developing protocols for steady-state fermentation of A. niger under repressing conditions for the glaA promoter and for transition to a glaA induced steady-state. The samples collected throughout these fermentations are used for transcriptomic and protein analysis.
Tools are developed to improve the genetic screen for the isolation of regulatory mutants with altered UPR characteristics.
We will report on the recent progress made in these project goals.
This research is carried out within the Kluyver Centre for Genomics of Industrial Fermentation.

156. Comparative fungal genomics using the MIPS comprehensive resources. U. Gueldener[1,*], M. Muensterkoetter[1], G. Mannhaupt[2], H.W. Mewes[1,3]. [1]MIPS-Munich Information Center on Protein Sequences, GSF - National Research Center for Environment and Health, Ingolstädter Landstrasse 1, D-85764 Neuherberg, Germany. [2]Max Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl-von-Frisch-Strasse, D-35043 Marburg, Germany. [3]Technische Universität München, Chair of Genome Oriented Bioinformatics, Center of Life and Food Science, D-85350 Freising-Weihenstephan, Germany

The number and quality of currently sequenced fungal genomes is far beyond what has been expected some years ago. Having 30 analyzed fungal genomes at hand, the transfer of knowledge from the well studied model species *Saccharomyces cerevisiae* and *Neurospora crassa* to the fungal key target organisms like *Fusarium graminearum*, *Ustilago maydis* or *Phanerochaete chrysosporium* will be the main challenge in the near future. Extensive comparative analysis methods not only provide more information, but in turn allow the annotation of distinct functions to up to now unknown proteins and thus significantly improve the quality of the genome annotation. Moreover comparative analysis on protein domain level and synteny between fungal as well as fungal and other species groups reveal evolutionary coherences and putative horizontal gene transfer. Mapping and comparison of protein-protein interactions (interologs) help to imagine interactomes. Recently intra- and inter-genomic promoter comparisons were started which, in combination with expression data, will give insight into regulatory networks. First comparative analysis results will be presented.

157. Genomic analysis of the secretion stress response in *Aspergillus niger*. Thomas Guillemette[1], Noel van Peij[2], Karin Lanthaler[3], Geoff Robson[3], Hein Stam[2], David Archer[1]. [1]School of Biology, University Park, University of Nottingham, Nottingham NG7 2RD, UK. [2]DSM (Food Specialities), P.O. Box 1, 2600 MA Delft 624-0295, The Netherlands. [3]The Victoria University of Manchester, School of Biological Sciences, 2205 Stopford Building, Oxford Rd, M13 9PT Manchester, UK

Filamentous fungi such as *Aspergillus niger* have a well developed and high capacity secretory system and are therefore widely exploited for the industrial production of native and heterologous proteins. However, in most cases the yields of non-fungal proteins are significantly lower than those obtained for fungal proteins. One well-studied bottleneck appears to be the result of mis-folding of heterologous proteins in the ER during early stages of secretion, with related stress responses in the host, including the unfolded protein response (UPR), ER-associated (protein) degradation (ERAD) and a more recently discovered transcriptional repression of genes during secretion stress (RESS). The aim of our project is to use genomic approaches to gain insights into the bottlenecks that limit the application of *A. niger* as a host for recombinant protein production. A global analysis of secretion-related stress responses was performed using Affymetrix chips based on the sequenced genome available from DSM. In our experimental conditions, secretion stress in A. niger was induced by three means: (i) treatment of wild-type mycelium by chemical inducers of the UPR (DTT and tunicamycin), (ii) over-expression of a heterologous protein (t-PA), (iii) reduction of the level of ER-resident proteins involved in folding (PdiA). Overlapping (common to all conditions) and specific responses will be described in relation to secretion stress.

158. Characterization of the genes encoding tropomyosin and ARP4 in *Neurospora crassa*. Nahideh Haghighi[1], P. John Vierula[1]. [1]Biology Department, Carleton Uinversity, Ottawa, Canada.

This study has focused on the genes encoding tropomyosin and ARP4 in the filamentous fungus *Neurospora crassa*. Tropomyosin is an extended coiled-coil protein that binds to actin filaments and influences many aspects of F-actin. Repeat Induced Point (RIP) mutagenesis was used to produce a tropomyosin mutant strain. Mating with strains containing multiple copies of the *tpm* gene produced 20% inviable ascospores and approximately 7% of all spores exhibited an abortive germination phenotype. Only one out of 120 viable progeny displayed a mutant phenotype suggesting that the *N. crassa tpm* gene is essential for germination of ascospores. Moreover, scanning electron microscopy (SEM) and light microscopy of the *tpm*-RIPed strain showed that it branches more frequently than wild type. Staining with FITC labelled mouse a-actin did not detect an apical actin distribution, instead actin positioned predominantly in subapical zones.
The actin related proteins (ARPs) have primary sequence homology to actin. In the present study, an ARP in *N. crassa* was identified that has 30-35% amino acid identity to ARP4 from other species. Like other ARP4s, NcARP4 has a nuclear localisation signal (NLS) suggesting that it is taken up by the nucleus. One morphological mutant strain was detected among 90 RIP mutagenesis progeny and its *arp4* gene had very few G to A substitutions, suggesting that *arp4* is also an essential gene. Approximately, 20% of the *arp4*-RIPed germlings have nuclei localized at the extreme tips of hyphae, in comparison to the 8-10 mm distance of the apical nucleus in wild type.

159. The utility of the incomplete genome: the *Amanita bisporigera* genome project. Heather E. Hallen[1] & Jonathan D. Walton[2]. [1]Department of Plant Biology, and [2]DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

As part of ongoing investigations into the ecology, biochemistry and evolutionary biology of *Amanita* section *Phalloideae*, we have initiated the *Amanita* genome project. This project originated as part of our investigations into amatoxin biosynthesis. Amatoxins are presumed to be synthesized via a non-ribosomal peptide synthetase (NRPS), predicted to be encoded by a 30-kb gene. Assuming random sampling across the genome and an average read size of 600 bp, the odds of hitting a 30 kb target in a 40 Mb genome are high: 95% chance in 4,000 random, independent sequences. Consequently, we are generating several thousand genomic sequence reads from *Amanita bisporigera* (10,000+ as of November 30, 2004). Sequencing an EST library was impractical, as amatoxin biosynthesis appears to take place in a narrow window at or near the time of button initiation, and transcription of amatoxin biosynthetic genes is therefore not observable in the macroscopic organism. We have a generated a public *Amanita* sequence database. Each sequence has been compared to GenBank's non-redundant database (using BLASTX) and the *Coprinus* and *Phanerochaete* genomes (using TBLASTX). Additionally, we maintain all sequences in BLAST-searchable format. This resource is available at . We envision it being of particular interest to the ectomycorrhizal research community.

160. Fungal and plant gene expression in endophyte-infected plant tissues. U. Hesse and C. L. Schardl. Plant Pathology Department , University of Kentucky

Analysing gene expression of symbiotic microorganisms in-planta is difficult due to relatively low biomass. We investigated the fungal endophyte *Epichloë festucae*, which lives in symbiosis with cool-season grasses. It represents an interesting model organism for studying plant-microbe interactions because it develops mutualistic, parasitic, and pleiotropic relationships with its host plants. For most of the life cycle, the endophyte grows benignly through the intercellular spaces of the above-ground plant organs (pseudostems, leaf blades, inflorescences, seeds) without damaging or entering host cells. Mutualistic effects are associated with production of bioprotective alkaloids by the fungus, and positive effects on the drought tolerance of the host. Reproductive development of the host induces production of external hyphae and development of a stroma (a compact mycelial layer which serves as a cradle for ascogenous perithecia) enveloping the inflorescence. In parasitic *Epichloë* sp.-grass interactions host reproduction is virtually terminated and the rare seeds from escaped inflorescences are endophyte-free. *Epichloë festucae* is an intermediate: it exhibits both transmission modes (benign colonization of seeds and stromata production) on one plant. Sequencing of normalized vs. non-normalized cDNA libraries from endophyte-infected plant tissues, and genomic DNA of the fungus allowed extensive identification of expressed fungal and plant unigenes, which can be used in future hybridization studies for discovery of differentially expressed symbiosis genes.

161. Live cell imaging of fungal hyphae using fluorescent dyes and recombinant probes. Patrick Hickey[1] and Nick Read[2]. [1]LUX Biotechnology Ltd. Edinburgh, Scotland, EH9 3JL. [2]University of Edinburgh, Scotland, EH9 3JH.

Recent developments in imaging technology have allowed us to explore new dimensions in cell biology. Using confocal microscopy we can simultaneously image living fungal hyphae capturing up to three fluorescent channels and a bright field image. In addition to recombinant probes like GFP, a number of different fluorescent dyes have been utilised to target organelles and components of fungal hyphae. The importance of live cell analysis is discussed and an overview of the techniques required for image processing and time lapse animation is provided. Methods for optimising image quality, and reducing photobleaching and phototoxicity are described. Finally, the use of fungi in high throughput imaging applications is introduced.

162. Transcription analysis of carbon repression of *Aspergillus nidulans* using High Density Microarrays. Jesper Mogensen[1], Henrik Bjørn Nielsen[2], Gerald Hofmann[1], and Jens Nielsen[1] . [1]Center for Microbial Biotechnology, BioCentrum, DTU, Denmark. [2]Center for Biological Sequence Analysis, BioCentrum, DTU, Denmark

Glucose repression has been the subject of fungal research for many years now, and still there is a lot to be resolved, for example the many pleiotropic effects of mutations of the *creA* gene. Therefore this study aims at a better understanding of glucose and/or carbon repression in *A. nidulans*, utilising high density DNA-microarrays for high throughput transcriptional analysis.
The effect of CreA was investigated by comparison of the gene-expression of the *creA* mutant against a reference strain under growth conditions considered to be repressing (glucose) and de-repressing (ethanol) . Statistical analysis based on biological triplicates showed 200 significantly regulated genes (p-value less than 1.0×10^{-2}). Cluster analysis, based on the expression profiles of all four conditions, reveals a number of complex patterns, whose interpretation is hampered by the relative poor annotation of the majority of open reading frames (ORFs) the *A. nidulans* genome sequence. On the other hand, this enhances the value of this study, since it increases the knowledge about putative roles/functions of ORFs.
For further analysis and interpretation of the data, promotor analysis has been performed and the response of metabolic genes has been compared with data from C^{13} metabolic flux analysis of the same *creA* mutant strain.

163. Evolutionary differences in the fungal carbamoyl-phosphate synthetase small subunit gene and associated upstream open reading frame. Heather M. Hood and Matthew S. Sachs. Oregon Health & Science University, Beaverton, OR 97006

*Neurospora crassa arg-2*and *Saccharomyces cerevisiae CPA1* encode the small subunit of arginine-specific carbamoyl-phosphate synthetase (CPS-A), which is required for arginine biosynthesis. Excess arginine negatively regulates the translation of each mRNA. This is accomplished via the action of a 5' upstream open reading frame (uORF) that encodes a *cis*-regulatory element, the arginine attenuator peptide (AAP). The AAP stalls ribosomes in response to arginine and by doing so reduces synthesis of the enzyme, which is produced from a downstream open reading frame. Here we show a comparative analysis of the genes and predicted regulatory elements of *arg-2* homologs obtained from genomic sequences of Ascomycetes and Basidiomycetes. The AAP peptide and the structures of the genes specifying CPS-A has diverged between these phyla. Major differences detected included variation in intron number and position and varying degrees of regulatory motif conservation. Interestingly in *Kluyveromyces lactis*, the reading frames encoding the AAP and the CPS-A enzyme overlapped. Despite many differences across species, there are three perfectly conserved amino acid residues in the predicted AAP among all fungi, including an aspartic acid known to be crucial for arginine-dependent regulation of *arg-2* and *CPA1*. We are following up on these *in silico* results by testing the efficiency with which the different isoforms of the AAP exert translational control.

164. Transcriptional Responses to Secretion Stress in the Fungi Trichoderma Reesei and *S. cerevisiae* Reveal Interesting

Differences and Common Features. Mikko Arvas, Tiina Pakula, Karin Lanthaler*, Geoff Robson*, Markku Saloheimo and Merja Penttilä. VTT Biotechnology, Tietotie 2, Espoo, PL 1500, 02044 VTT, Finland. *Univ. of Manchester, Fac. of Life Sciences, 1800 Stopford Building, Oxford Road, Manchester M13 9 PT, UK.

Trichoderma reesei is an industrial protein production host known for its exceptional protein secretion capability. This study aims at uncovering the transcriptional responses occurring in *T. reesei* cells exposed to secretion stress and comparing these responses to similar experiments carried out in *S. cerevisiae*. Secretion stress is caused by compromised protein folding or transport in the secretory pathway. It induces a number of genes involved in different aspects of secretion through the unfolded protein response (UPR) pathway. In T. reesei it has also been shown that secretion stress down-regulates genes encoding secreted proteins.
We constructed cDNA subtraction libraries and made cDNA-AFLP (amplified fragment length polymorphism) experiments from cells under secretion stress. A transformant expressing human tissue plasminogen activator (tPA), treatment with the chemical DTT (dithiothreitol) that prevents correct protein folding and a transformant over-expressing IREI protein (sensor protein of the UPR pathway) were analysed. Around two hundred unique ESTs were retrieved by these methods and the expression pattern of about 50 was confirmed by Northern experiments. A rank sum test for the Northern data was used to define those genes that show upregulation in all the three conditions. Data from DTT and tunicamycin treatment, foreign protein production and IRE1 and HAC1 (UPR transcription factor) deletion experiments in S. cerevisiae were combined from litterature. The transcriptional responses of *T. reesei* and *S. cerevisiae* show clear overlap, especially with respect to genes involved in protein translocation, folding and glycosylation in the ER. However, there seems to be differences in regulation of amino acid biosynthesis and nucleosome genes. The GCN4/CPC1 transcription factor and a limited set of its putative target genes are induced in T. reesei. This response points to the upregulation of glutathione synthesis to relieve oxidative stress caused by compromised protein folding. Interestingly also a set of nucleosome genes is upregulated in T. reesei without a clear connection to cell cycle.

165. A proteomic approach to identify proteins secreted by *Fusarium oxysporum* in xylem sap of tomato.. Petra M. Houterman[1], Dave Speijer[3], Henk L. Dekker[2], Ben J.C. Cornelissen[1] and Martijn Rep[1]. [1]Plant Pathology, [2]Mass Spectrometry, Swammerdam Institute for Life Sciences, [3]Medical Biochemistry, Academic Medical Center (AMC), University of Amsterdam, The Netherlands.

Fusarium oxysporum f. sp. *lycopersici* is a soil-born fungus that causes vascular wilt disease in tomato. *Fusarium* invades the plant via the roots and subsequently colonizes the plant via the xylem vessels. Proteins that play an important role in the interaction between plant and pathogen are therefore likely to be secreted into the xylem sap.
We analyzed the proteins that accumulate in the xylem sap of tomato upon infection with *F. oxysporum*. These proteins were identified using a combination of 2-dimensional gel electrophoresis (2-DE), peptide mass fingerprinting (MALDI MS) and mass spectrometric sequencing (LC MS/MS).
We had shown earlier that a number of pathogenesis related (PR) proteins from tomato and SIX1 (secreted in xylem 1) from *Fusarium* accumulate in xylem sap after infection (1,2). Here we report the identification of additional plant and possible fungal proteins. From tomato we identified a polygalacturonase (endoPG), a number of peroxidases, a xylogucan-specific endoglucanase inhibitor protein (XEGIP) and a xyloglucan endotranglycosylase (XET). A number of sequence tags were obtained from unidentified, possibly fungal, proteins. These will enable us to use degenerated PCR for identification of coding sequences.
(1) Rep et al. (2002) Plant Physiology 130: 904-917. (2) Rep et al. (2004) Molecular Microbiology 53(5): 1373-1383.

166. Gene disruption in *Cryptococcus neoformans* by *in vitro* transposition. G. G. Hu and J.W. Kronstad. Michael Smith Laboratories, The University of British Columbia, Vancouver, B.C. V6T 1Z4

Cryptococcus neoformans is a basidiomycetous fungus that infects immunocompromised and immunocompetent patients. Five serotypes (A, B, C, D and AD) have been documented based on antigenic differences in the polysaccharide capsule. In the present study, an *in vitro* transposon-based insertional mutagenesis strategy was developed and applied to *C. neoformans*. The goal was to establish a method to rapidly disrupt genes with interesting expression patterns, as determined by SAGE, or genes identified as part of our whole genome sequencing project for serotype B strains. To test the method, we disrupted the *URA5* gene in the serotype A strain H99 and the *CAP10* gene in the serotype B strains WM276 (VGI) and R265 (VGII). For the target DNA, we selected either plasmid DNA containing the cloned *URA5* gene, or plasmid DNA containing the *CAP10* gene from our 2 kb genomic library from the WM276 shotgun sequencing project. In the latter case, we have the end sequences of the 2 kb clones matched with the genomic sequence so that the clones for any gene can be selected for disruption. Modified transposons, containing the nourseothricin (*NAT*), neomycin (*Neo*), or hygromycin (*Hyg*) resistance cassettes, were randomly inserted into the target DNA (or gene) in an *in vitro* transposition reaction. Clones in which the gene of interest had been disrupted were identified by colony PCR and/or DNA sequencing and used to biolistically transform the strains of *C. neoformans*. Using this approach, we obtained H99 *ura5*:: *NAT* and R265 *cap10*:: *Neo* mutants. Confirmation and characterization of the mutants was performed by PCR, Southern blot and phenotype analysis. This technique was rapid and efficient for gene disruption compared to current strategies, and worked in a variety of strain backgrounds. The use of plasmid DNA from the shotgun-sequencing library combined with random *in vitro* insertions should allow high-throughout genetic analysis, particularly in serotype B strains of *C. neoformans*.

167. Haplotype comparison of an ultra gene dense island containing a G-protein Coupled Receptor gene in *Phytophthora infestans*. Iziah Sama, Keith O'Neill*, Francine Govers and Rays H.Y. Jiang. Plant Sciences Group, Laboratory of Phytopathology,

Wageningen University, and Graduate School Experimental Plant Sciences, The Netherlands and *Broad Institute, Cambridge, MA 02141, USA.

Phytophthora infestans is a notorious oomycete pathogen causing potato late blight world wide. An ultra gene dense region of 8.9 kb containing the G-protein Coupled Receptor gene *Pigcr1* is identified as a gene island in a BAC sequence comprising 107 kb. Haplotype comparison of the surrounding 40 kb region, using the sequence of a pair of allelic BACs, revealed very little polymorphism in this part of the genome. In addition to the high gene density (on average one gene per 1,8 kb) other remarkable features are found such as alternative splicing, shared core-promoter regions and overlapping 3' UTR's. To estimate the frequency of overlapping 3' UTRs in the *P. infestans* genome a bioinformatics tool was developed and a large *P. infestans* EST contig set was analysed. Synteny studies with the *Phytophthora sojae* and *Phytophthora ramorum* genomes demonstrated that the gene island is present in all three species and that the genes in this ultra gene dense region show a highly conserved order and orientation.

168. The external proteome of *Histoplasma capsulatum*. C Johnson[1], S Douangkesone, C Lichti, V Kruft, and J McEwen. J.L. McClellan VA Hospital, Little Rock, AR.

The human fungal pathogen, *Histoplasma capsulatum*, possesses a complex cadre of mechanisms for overcoming the immune system. Some of these are the ability to overcome the macrophage oxidative burst, to inhibit phagosome/lysosomal fusion and to regulate the internal pH of the phagolysosome. These abilities imply that *H. capsulatum* can regulate the conditions of its immediate external environment, possibly by secreting regulating molecules. In an effort to determine the composition of such an externally located regulating environment, we are characterizing the external proteome of *H. capsulatum*. To accomplish this, yeast proteins isolated from in vitro culture growth medium were resolved by 2-dimensional gel electrophoresis and the protein spots analyzed by mass spectrometry. Initial results indicate processed forms of the yps3 protein and *H. capsulatum* immunoreactive protein are part of the external proteome. Chitinase, in multiple modified forms, and the product of the catalase B gene, with a mass and amino terminus significantly different from that of the previously characterized mycelial M antigen (Zancope-Oliveira et al., 1999), also make up part of the yeast external proteome. As well, oxidative stress alters the composition of the external proteome by increasing the abundance of several of the proteome components, including the catalase B protein. In our earlier work, investigating gene expression at the level of transcription, we showed no significant increase in abundance of the catalase B transcript in yeast during oxidative stress (Johnson et al., 2002).
Our initial results show the external proteome of *H. capsulatum* has a number of secreted proteins in post-translationally modified forms. Chitinase is present in isoforms that differ by charge and mass and catalase B has a significant increase in mass. Furthermore, our findings show that regulation of catalase B gene expression is occurring at a post-transcriptional level under conditions of oxidative stress. Ongoing direct mass spectrometry analysis has identify additional components of the external proteome. Furthermore, we are attempting to define the specific post-translational modifications being made to the components of the external proteome.

169. Transcriptomics and metabolomics: An integrated approach to dissect endophyte-grass symbioses. Linda J. Johnson, Shalome Bassett, Gregory Bryan, Michael Christensen, Karl Fraser, Geoff Lane, Richard Johnson, Brian Tapper, Christine Voisey, Hong Xue, Susanne Rasmussen. AgResearch Grasslands, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand.

Grass associations with fungal endophytes (genera *Neotyphodium* and *Epichloë*), display enhanced fitness as well as prolonged field persistence over their endophyte free equivalents. In order to understand how the endophyte globally affects its host plant's metabolism, and to gain a holistic understanding of the complex biological interactions that occur between a plant host and fungal symbiont, a combined metabolomics and transcriptomics approach using microarrays is currently being employed. The major metabolites from symbiotic interactions of perennial ryegrass (*Lolium perenne*) with endophytes *N. lolii* and *E. festucae* are being compared and analysed against endophyte-free perennial ryegrass. Transcriptomic and metabolite data generated from the perennial ryegrass-*N. lolii* interaction clearly show that the endophyte does affect its host plant's metabolism and these data will be presented.
An additional goal is to elucidate the biosynthetic function of fungal secondary metabolite gene clusters since there are numerous examples from other plant-fungal interactions where host functions can be manipulated via the production of fungal secondary metabolites. For this study, we have selected non-ribosomal peptide synthetase genes (NRPSs) of unknown function, as these genes are commonly associated with the production of important bioactive peptides. The metabolome and transcriptome of *E. festucae* endophyte strains containing targeted gene replacements in two of these pathways will be compared with the corresponding isogenic wild type *E. festucae* strains under culture conditions as well as *in planta*. From the integration of transcriptomic and metabolomic data, we hope to identify key regulatory and biochemical networks responsible for the maintenance of these plant-fungal associations.

170. Proteomic analysis of regulation and signalling. Meriel G Jones, Igor Morozov, Alisha Millard, Huw H Rees, Deborah Wood and Mark X Caddick. School of Biological Sciences, The University of Liverpool, UK

Our work investigates regulatory responses to extracellular pH and the availability of nitrogen and phosphate in the filamentous fungus *A. nidulans*. All are essential biological processes and have been implicated in fungal pathogenicity. The related species *A. fumigatus* is an important opportunistic pathogen in immunocompromised patients but the biology of its multifactoral pathogenicity determinants is poorly understood. The combination of proteomics with the recently released genome sequences of both *A. nidulans* and *A. fumigatus*, combined with the genetic resources offered by *A. nidulans*, offers an opportunity to advance knowledge of these processes. Although the *pacC/pal* system is known to regulate response to pH in *A. nidulans*, the combination of proteomics and mutant strains can provide leads to the extent, and identity, of additional factors. We have examined the response of the intracellular soluble and external secreted proteomes of *A. nidulans* to external pH during growth. We are also interested in applying proteomics to investigate signalling mechanisms, by looking at the primary response to an environmental shift. We are examining the response of the proteome to altered nitrogen status within two minutes to identify early consequences of nitrogen metabolite signalling. We will present a brief description of the biology of the systems under study and our recent work.

171. Serial Analysis of Gene Expression in *Coccidioides posadasii.* Ellen M. Kellner[1,2,3,4], M. Alejandra Mandel[1,2,3,4], John Galgiani[1,3] and Marc Orbach[1,2]. [1]Valley Fever Center for Excellence and [2]Department of Plant Sciences, University of Arizona, and [3]Southern Arizona Veterans Administration Health Care System, Tucson, AZ. [4]These authors contributed equally to this work.

Coccidioides posadasii is a primary fungal pathogen endemic to the desert southwest US, parts of Mexico and Central and South America. Coccidioidomycosis or Valley Fever is initiated upon the inhalation of arthroconidia from the soil. *Coccidioides spp.* are dimorphic and produce unique structures that are characteristic of its saprobic and parasitic growth phases. Mycelia and conidia are formed in soil whereas the host-specific phase involves the growth of spherules and production of endospores. We are taking a genomic approach to identify expressed genes in mycelia and spherules of *C. posadasii* in order to identify cell-type specific genes. Parasitic phase-specific genes may include regulatory factors controlling spherule morphogenesis, novel targets for pharmaceutical intervention or potential vaccine candidates. Serial analysis of gene expression (SAGE) was used to create libraries of expressed tags from 48-hour liquid-grown mycelium at 37 ^{o}C and 48-hour liquid-grown spherules at 39 ^{o}C. The SAGE libraries each contain several million tags of which approximately 100,000 were identified through sequencing. The genome of *C. posadasii* strain C735 was used to examine the flanking regions of expressed sequence tags to identify potential genes. Tags that were found to be enriched in 48hr spherule library relative to the mycelial library included a laccase, an alkaline phosphatase and an L-ornithine N5-monooxygenase. Tags present in highest numbers in both mycelia and spherules included many that mapped to genes encoding histones and ribosomal proteins. Real time RT-PCR was used to examine the expression patterns of selected gene classes during growth and spherule development both to validate the SAGE library and to extend our analyses.

172. Analysis of gene expression in the entomopathogenic fungus *Beauveria bassiana*. Eun-Min Cho and Nemat O. Keyhani. University of Florida, Microbiology and Cell Science, Bldg 981, Museum Rd. Gainesville, FL 32611

Beauveria bassiana is an important entomopathogenic fungus that displays a broad host range able to target a diverse number of arthropod species. Strains of *B. bassiana* have been selected for control of insects and other arthropods that act as disease vectors, crop and household pests, and have been used as biocontrol agents for limiting the spread of ecologically hazardous, invasive insects. *B. bassiana* produces at least three distinct single cell propagules including aerial conidia, vegetative cells termed blastospores, and microcycle conidia that can be isolated from agar plates, rich broth liquid cultures, and under conditions of nutrient limitation in submerged cultures, respectively. cDNA libraries were constructed from each *B .bassiana* cell type and a robust expressed sequence tagged (EST) dataset was generated. Approximately 2,000 clones from each library were sequenced and a unique sequence set was constructed. Comparative analysis of the expressed transcripts in each library indicated significant differences in gene expression pattern between the cell types in several broad categories including cell wall biosynthesis, secondary metabolism, and the production of proteases.
This work was supported in part by NSF grant # EF-0412137 (NOK).

173. Temperature-dependent Gene Expression in Aspergillus fumigatus Examined by DNA Microarray. H. Stanley Kim[1], Dan Chen[1], Christine E. Shamblin[1], Gregory S. May[2], Michael Anderson[3], and William C. Nierman[1]. [1]The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20854, USA. [2]The University of Texas M. D. Anderson Cancer Center, Houston, TX USA. [3]University of Manchester, Oxford Road, Manchester M13 9PT, UK

Recent completion of the genome sequencing of the opportunistic human pathogen Aspergillus fumigatus provided a great opportunity to study its biology in greater detail. One of the characteristic phenotypes of A. fumigatus is its high adaptability to elevated environmental temperatures not tolerated by other members of the species. These environments, including soil, animal hosts, and compost, requires the fungus to adjust its biochemical constitution and physiology to survive and possibly resume growth after exposure to a temperature as high as 70o C. We examined genome-wide expression upon temperature shift from low to high and from high to low, within the range of 30¢ªC to 52¢ªC. We found that different genes have specific windows of expression over the temperature range and the duration at a given temperature. For example, many heat shock and stress-responsive genes were immediately up-regulated when the culture was shifted to high temperatures (above 48¢ªC), but some of these were also up at 37¢ªC after about an hour. On the other hand, many putative virulence genes were readily expressed at 37¢ªC, as if the fungus were in the host. This suggests that temperature change is an environmental signal by which the fungus adjusts its physiology in an environment appropriate manner. Another finding we made from this study is that many transposases, especially those of the Mariner-4 type, are highly expressed at high temperatures. This suggests that transposition might be activated at high temperatures perhaps as a means to allow improved adaptability to the high temperature stress through genome alterations. We are extending this study relating the temperature-responsive genes to the Comparative Genomic Hybridization (CGH) data with closely related stains.

174. The *Alternaria brassicicola* Genome Sequencing Project. La Rota C. Mauricio [1], Brett Tyler [1], Thomas Mitchell [2], Susan Brown[3], Dennis Knudson [3], Sandra Clifton [4] and Christopher Lawrence [1]. [1]Virginia Bioinformatics Institute, VPI & State University, Blacksburg, VA. [2]NC State University, Raleigh, NC. 3. Colorado State University, Fort Collins, CO. 4. Washington University Genome Sequencing Center, St. Louis, MO.

This project aims to produce a draft genome sequence resource for the necrotrophic fungus, *Alternaria brassicicola*. *A. brassicicola*, with a predicted genome size of 30 Mb, is the causal organism of black spot disease and is an economically important pathogen of Brassicas. Moreover, *A. brassicicola* is representative of a genus of pathogens that inflicts substantial damage worldwide. The genus Alternaria as a whole has a dramatic impact on humans and human affairs, as it is also associated with mycotoxin contamination of food and food products, allergy, asthma, and opportunistic infection of immuno-suppressed patients. Importantly, *A. brassicicola*, which naturally infects cruciferous plants, has been used consistently as a model necrotrophic pathogen of Arabidopsis. No publicly available genome sequence exists for any true necrotrophic fungus that has a corresponding host genome publicly available. Excellent structural and functional genomic resources for *A. brassicicola* are currently being developed. In this project, the Washington University Genome Sequencing Center (WUGSC) will produce a whole genome shotgun sequence (WGS) of *Altenaria brassicicola* to 6-fold coverage. A BAC library will be used to generate a BAC-based fingerprint physical map of the genome. Assembly of the BAC library into contigs will be completed using restriction enzyme fingerprinting, and a minimum tile of BACs will be end-sequenced to provide a framework to facilitate assembly. A 0.3-fold coverage fosmid library will also be constructed and end sequenced at the WUGSC, as an additional resource to benefit assembly. Massively parallel signature sequencing (MPSS) will be used as an additional annotation feature for gene prediction. A bioinformatics platform for community finishing and annotation of the draft sequence will also be established. This project is funded by USDA-CSREES (Proj. # VAR-2004-05551).

175. Transcriptional Analysis of the Pathogenic Fungus *Magnaporthe grisea* During Rice Infection. Arnaud Lagorce*, Aurélie Darchis*, Fabien Munier*, Jean-Benoit Morel**, Rick De Rose*, Roland Beffa* et Marc-Henri Lebrun*. *Bayer CropScience / CNRS, 69263 Lyon Cedex 09, France. ** CIRAD, TA73/09, 34398 Montpellier Cedex 05, France

Magnapothe grisea – Oryza sativa is a relevant model to investigate the molecular mechanisms underlying fungal infection of plants. *M. grisea* infections are responsible of the main foliar disease of rice and cause severe crop losses world-wide. This fungus is well suitable to molecular biology since most genomic tools are available. Using microarrays representative respectively of 14 000 ORF from *M. grisea*, we performed a genome - wide transcriptional analysis to highlight fungal genes expressed during the infection process. Leaves from the resistant rice cultivar IR64 carrying the resistance gene Pi33 were infected by the virulent *M. grisea* strain PH14. Infected rice leaves were harvested 5 days after infection when the first lesions appear. This time point corresponds to the active invasion and destruction of plant tissues by *M. grisea*. Out of the *M.grisea* 14 000 genes, we could detect hybridization signals for 3000 genes that are expressed during infection. The expression of a subset of genes that are either (1) highly expressed during infection or (2) are expressed during infection but not in the mycelium was monitored using real time RT-PCR. This strategy confirmed the expression during infection of 75% of these genes. Additionally, the expression of these genes was monitored in different fungal tissues (mycelium, spore, appressorium) revealing 8 genes that are only expressed during infection. These data were used for clustering analysis revealing 4 major expression groups (constrictively expressed, infection specific, spore/infection specific, spore/appressorium/infection specific).
In order to assess the role of these genes in the infection process, *M. grisea* mutants will be constructed by gene replacement or RNA interference. RNA interference constructs expressing hairpin structures under the control of the promoter from the highly expressed genes *MPG1* were generated to inactivate known pathogenicity genes such as *PLS1* and *BIP1*. Whereas, deletion mutants obtained by gene replacement of *PLS1* or *BIP1* are completely non-pathogenic, their inactivation by RNA interference only reduces the pathogenicity of the transformants by 50 to 90%. These observations illustrate the difficulty in turning completely off gene expression during infection using RNA interference.

176. Genome sequencing of the arbuscular mycorrhizal fungus *Glomus intraradices*. Peter J. Lammers[1,2], Swarnamala Ratnayaka[2], John B. Spalding[2], Jeongwon Jun[2], Tarik Ceylon[1], Ian R. Sanders[3], J. Chris Detter[4]. [1]Dept. Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA. [2]Molecular Biology Program, New Mexico State University, Las Cruces, NM 88003 USA. [3]Dept. Ecol & Evol, University of Lausanne, 1015 Lausanne, Switzerland. [4]DOE Joint Genome Institute, Walnut Creek, CA 94598, USA

Arbuscular mycorrhizal (AM) fungi are coenocytic, multinucleate, asexual, obligate symbionts that colonize the majority of plant species. Approximately 150 species of AM fungi colonize over 200,000 species of plants resulting in a 5%-20% increase in photosynthesis and a large, if poorly understood contribution to the global carbon cycling budget. A genome sequencing project was funded by the DOE for a model AM fungus. *Glomus intraradices* was chosen because it a) has a worldwide distribution b) colonizes both annual and perennial plants of economic importance including maize, wheat, alfalfa, rice and the poplar tree c) can be grown aseptically in dual culture with Ri-transformed carrot roots and d) spores of this species are available commercially in large quantities (Premier Tech, Quebec Canada). Approximately 25 million base pairs of single-pass, unassembled sequence data was available by early December 2004 plus 3,500 pre-existing EST sequences. Key genes identified in the preliminary data include nutrient transporters, cell signaling and cell cycle regulation proteins, transcription factors, RNA polymerase subunits and splicing factors. An unexpected yet significant match was also found to a meiosis-specific recombination protein from yeast. Comparison of the genomic sequences with a mixed EST data set derived from the model legume *Medicago truncatula* colonized with *Glomus versiforme* was used to identify fungal genes expressed in symbiotic root tissue. Several dozen putative AM fungal sequences expressed in symbiotic root tissue were identified. Particularly well represented in the mixed EST database were Rab-like sequences highly similar to fungal proteins involved in protein trafficking and secretion.

177. Exploring new genomes, unveiling new enzymes: description of a new type of multicopper oxidase in *Phanerochaete chrsysosporium* and other fungal genomes. Luis. F. Larrondo[1], Rafael Vicuna[1] and Dan Cullen[2]. [1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile and Millenium Institute for Fundamental and Applied Biology.[2]USDA Forest Products Laboratory, Madison, Wisconsin 53705, USA

Laccases belong to the multicopper oxidase family, which also contains ascorbate oxidases, transmembrane Fet3 ferroxidases and ceruloplasmin. Until not long ago it was accepted that the only members of this family capable of oxidizing iron were fungal Fet3s and ceruloplasmin. It was recently shown that *Phanerochaete chrysosporium*, unlike other lignin-degrading fungi, does not have any sequence encoding conventional laccases (Larrondo et al., AEM 69: 6257-6263 2003). Instead it produces a novel multicopper oxidase possessing strong ferroxidase activity with catalytic parameters similar to those of yeast Fet3p. The physiological function of this protein (MCO1) is still uncertain.
The gene (*mco1*) is part of a cluster of 4 structurally related sequences located within a 25 kb region. All 4 genes are transcribed, but only mco1 has a clear secretion signal.
Multiple alignments of a large collection of fungal multicopper oxidase sequences, as well as structural comparisons of MCO1, show that these novel MCOs are closer to Fet3 proteins, than to conventional laccases. Together with iron permease Ftr1, Fet3 ferroxidase plays a key role in iron homeostasis. Clustal analysis of multicopper oxidases sequences from *P. chrysosporium, Magnaporthe grisea, Fusarium graminearum* and *Ustilago maydis* databases supports the existence of a new branch of the multicopper oxidase family. These fungal sequences group together, distinguishing themselves from the classical laccases or Fet3-ferroxidases by important features including: 1. the presence of the residue equivalent to Glu-185 from *S. cerevisiae* Fet3, which has been shown to be necessary for ferroxidase activity and; 2. the absence of a C-Terminal transmembrane domain, which is characteristic of Fet3-ferroxidases.
Based on the strong ferroxidase activity presented by *P. chrysosporium* MCO1, and by the presence of these sequences in plant-associated fungi genomes (not present in *S. cerevisiae, C. albicans, N. crassa, P. anserina* etc), we have hypothesized a possible role for this new type of enzyme in modulating redox reactions. Future studies will clarify the role and physiological significance of this new enzyme family.

178. An experimental screen for non-neutral evolving genes in symbiotic fungi. Le Quéré, A., Astrup-Eriksen, K., Rajashekar, B., Schützendübel, A., Canbäck, B., Johansson, T. and A. Tunlid. Lund University, Microbial Ecology, Lund, Sweden

Background: Ectomycorrhizae are formed by mutualistic interactions between fungi and the roots of woody plants. During the symbiosis, the two organisms exchange carbon and nutrients. Many fungal species are known to form ectomycorrhizae and there is a large variation in their host preferences and growth characteristics. The genomic basis for symbiotic adaptations and related phenotypic variations is poorly understood.
Results: The genomes of strains of the ectomycorrhizal fungus *Paxillus involutus* were compared using cDNA microarrays. A simple relative rate test was developed to identify genes that have evolved at a different rate within *Paxillus* as compared with the overall evolutionary rate in homobasidiomycetes. Approximately 17% of the genes were detected as non-neutral evolving in *Paxillus*. The cohort identified showed an overrepresentation of orphans, genes whose products are located at membranes, and genes encoding for components of stress/defense reactions.
Conclusions: We propose that data obtained from microarray-based comparative genomic hybridisation in combination with data on sequence divergence of the printed genes can be used for screening genomic variations associated with adaptive evolution. Using this approach, a subset of genes was identified in *P. involutus* that have an enhanced rate of evolution. Accelerated evolution may reveal functional differences in the corresponding proteins which are associated with symbiotic adaptations.

179. Proteomic analysis of *Monascus* strains reveals proteins associated with pigment production. Jo-Chi Wang, Huan-Yu Lin, Li-Ming Sung, Li-An Lai, Lina Huang, and Wen-Shen Chu. Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, ROC

Monascus fungi have been used for preparation of oriental fermented foods for centuries. They are rich sources of various secondary metabolites, such as pigments, antibiotics, mycotoxin and enzyme inhibitor. Organisms in the genus *Monascus* produce a mixture of six major pigments of polyketide origin. In addition to the well-known utilization as natural colorants, the pigments have been reported to exhibit some biological activities including antibacterial, antifungal and immunosuppressive effects. However, the mechanism of pigmentation has yet to be elucidated. A proteomic analysis of a non-pigment producing mutant, *Monascus purpureus* BCRC 38112, along with its parental strain *Monascus purpureus* BCRC 31499 having good red pigment production ability has been performed to investigate proteins related to pigment production. Proteomes of the two strains were separated by one- and two-dimensional electrophoresis followed by image analysis. A total of 80 protein spots with significantly different expression level were obtained. These protein spots were identified by tandem mass spectrometry and Mascot search system. Important qualitative differences between the strains were found in proteins involved in fatty acid metabolism, carbohydrate metabolism, ATP biosynthesis, protein folding and protein processing. This study shall provide useful information for further analysis of pigment production in *Monascus* spp..

180. A High Density SNP map for *Neurospora crassa*. Mi Shi[1], William Belden[1], Arun Mehra[1], Allan Froehlich[1], James E. Galagan[2], Bruce W. Birren[2], Jay C. Dunlap[1], and Jennifer J. Loros[3]. [1]Dept. of Genetics, Dartmouth Medical School, Hanover, NH 03755; [2]Broad Institute, Cambridge, MA 02141; [3]Dept. of Biochemistry, Dartmouth Medical School, Hanover, NH 03755.

The *Neurospora crassa* genome contains about 2000 map units. As a rule of thumb there are about 20 kb per 1% recombination in the midst of chromosome arms, suggesting that the population of a thousand SNPs on the Neurospora genome would allow rapid reduction of genetic map location to physical sequence of novel genes. In pursuit of this goal, we have compared sequences from two isolates of *N. crassa* – the Oak Ridge laboratory "wild-type" used as the basis for the genomic sequence completed in 2001 (Galagan et al Nature 422, 859 – 868, 2003) and the *N. crassa* isolate from Mauriceville Texas used previously as the basis for a widely used RFLP map (Metzenberg et al. Proc. Natl. Acad. Sci. U.S.A. 82:2067-2071) – and at this writing have populated a map with several hundred SNPs, a number we expect will soon exceed a thousand.

Sequencing of cDNA libraries made from FGSC2225 (*N. crassa* Mauriceville, A) has revealed SNPs at a raw frequency of approximately 5 per 1000 nucleotides. These comprise the bulk of the newly identified SNPs. Additionally, we have focused attention on the centromeric region of LG 1 and on LG IV, deriving additional SNPs from directed local sequencing of FGSC 2225 DNA based on the available genomic sequence. In all cases, SNPs are detected amongst cross progeny using a PCR-based strategy (http://ausubellab.mgh.harvard.edu). Three oligos are needed to differentiate one SNP with certainty. Use of SNPs on LG IV has allowed the improvement of the current genome assembly, reversing the orientation of one supercontig as well as placing previously unordered sequences. Additionally, the SNPs have allowed the physical cloning of several genes of interest.

181. Development of a Genetic Linkage Map of *Colletotrichum Lindemuthianum*. F. Luna-Martinez[a], R. Rodriguez-Guerra[b], M. Victoria Campos[a] and J. Simpson[a]. [a]Department of Genetic Engineering, CINVESTAV, Unidad Irapuato, Apdo. Postal 629, Irapuato, Guanajuato, Mexico; [b]Instituto Nacional de Investigaciones forestales, Agricolas y Pecuarias (INIFAP), Campo Experimental del Bajio, Celaya, Guanajuato, Mexico.

A genetic map was being developed for *C. lindemuthianum* (Sacc. & Magnus) Lambs.-Scrib, the causal agent of anthracnose in Bean (*Phaseolus vulgaris*). Although classified as Deuteromycete, we have obtained a segregating F1 population of *C. lindemuthianum* from a sexual cross produced under laboratory conditions. We are currently developing a genetic map of the fungus using AFLP molecular markers. Currently the map is based on 49 F1 progeny derived from a cross between two Mexican isolates. A total of 131 markers have been mapped, covering 937 cM and spread over 14 major linkage groups. Significant deviation from the expected 1:1 segregation ratios was observed for only 16 markers (prob. of X^2). C. lindemuthianum and will open the possibility to study the genetics of mating type specificity and avirulence factors in this pathogen.

182. Characterization of *Aspergillus oryzae* genome structure. Masayuki Machida[1], Motoaki Sano[1], Kiyoshi Asai[1], Toshitaka Kumagai[1], Taishin Kin[1], Hideki Nagasaki[1], Goro Terai[2], Takashi Komori[2], Toshihiro Tanaka[3], Rie Igarashi[3], Toshihiko Sawano[3], Hisashi Kikuchi[3], Toshihide Arima[4], Osamu Akita[4], Kenichi Kusumoto[5], Yutaka Kashiwagi[5], Keietsu Abe[6], Katsuya Gomi[6], Michio Takeuchi[7], Tetsuo Kobayashi[8], Hiroyuki Horiuchi[9], Katsuhiko Kitamoto[9], James E. Galagan[10], David W. Denning[11], William C. Nierman[12], Jennifer Wortman[12], Jiujiang Yu[13], Deepak Bhatnagar[13], Thomas E. Cleveland[13], A. oryzae genome sequencing consortium of Japan[14]. [1]Natl. Inst. Advanced Ind. Sci. Tech. [2]INTEC Web and Genome Informatics, [3]Natl. Inst. Tech. Eval., [4]Natl. Res. Inst. Brewing, [5]Natl. Food Res. Inst., [6]Tohoku U., [7]Tokyo U. Agric. Tech., [8]Nagoya U., [9]U. Tokyo, [10]Broad Inst. MIT & Harvard, [11]U. Nottingham, [12]The Inst. Genomic Res., [13]South Regional Res. Center, [14]Brewing Soc. Japan.

The genome sequencing of *Aspergillus oryzae* (AO) was completed. The total AO genome size was 36.8 Mb, from which more than 11,000 genes were predicted. Synteny analysis among AO, *A. fumigatus* (AF) and *A. nidulans* (AN) showed that AO has significantly more synteny breaks than between AF and AN and that AO genome had a mosaic structure consisting of AO-specific and common loci among the three species. Interestingly, metabolic genes were highly condensed on the AO-specific loci although they were repressed by mapping of ESTs. These results together with the existence of a pair of highly homologous and adjacently located genes in AO and *Agrobacterium tumefacience* suggest that AO might acquired foreign genes after it branched from AF and AN.

183. Development of an EST resource for the phytopathogenic oomycete *Aphanomyces euteiches*. Arnaud BOTTIN, Christophe JACQUET, Hélène SAN-CLEMENTE, Bernard DUMAS, and Elodie GAULIN. Université Paul-Sabatier, UMR5546 UPS-CNRS, Pôle de Biotechnologie Végétale, BP17, 31326 Castanet-Tolosan, FRANCE

The *Aphanomyces* genus is composed of saprophytic as well as parasitic species affecting either plant or animal hosts. Comparative genomics between *Aphanomyces* species, or between *Aphanomyces* and other oomycetes (e.g. *Phytophthora*), should help to identify the genes required for pathogenicity on plants or animals. *Aphanomyces euteiches* is a major root pathogen of Pea and Alfalfa in Northern America, and of Pea in Europe. Neither effective chemicals nor resistant cultivars are available to control the disease in Pea. In order to study the molecular basis of pathogenicity in *A. euteiches*, we are developping an EST sequencing project supported by the Genoscope d'Evry (France). Starting from mycelium grown in a standard medium or from starved mycelium placed in contact with host roots, two cDNA libraries are being constructed and 10.000 ESTs will be generated from each library. The sequences will be annotated and released on a publicly accessible web site hosted at our laboratory. Analysis of the sequences and of gene expression arrays will lead to a collection of putative pathogenicity genes. The functional characterization of these genes will involve their silencing after *Agrobacterium*-mediated transformation, which is under development at our laboratory. Progress of the project and preliminary results will be presented.

184. Gene modelling and Annotation of the complete *Ustilago maydis* Genome. Gertrud Mannhaupt, Ulrich Güldener and Werner Mewes. Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Our project was initiated to improve the predicted gene set of the *Ustilago maydis* genome, recently released by the Broad Institute. Manual inspection and correction are applied to set up a comprehensive *Ustilago maydis* Genome database. This will be done by comparing the predicted gene set with a set of gene prediction algorithms trained with various fungal coding sequences, available cDNAs, and a nonredundant protein database.
These combined data are graphically presented, enabling the annotator to retrieve information dynamically. Gene models will be changed according to cDNA matches and to obtain best homology to experimentally known proteins.
The protein set of the Broad Institute´s release as well as a protein set independently generated at MIPS are already analysed and presented using the PEDANT system. PEDANT is a fully automated system using a wide spectrum of sequence analysis and structure prediction tools.
In the course of the project MUMDB (Mips Ustilago Maydis Data Base) is already established. Both protein data sets mentioned above are loaded into MUMDB omitting identical copies as well as all proteins derived from manually changed gene models. Beside annotating different features like INTERPRO, transmembrane domains or TargetP the proteins will be classified and assigned to functional categories. MUMDB will enable in depth intergenomic comparative analysis, especially using our recently developed SIMAP database containing all available protein sequences.

185. Cross species gene discovery using microarray analysis allows for the identification of D-galacturonic acid utilization pathway in *Aspergillus niger* and *Aspergillus nidulans*. Elena Martens-Uzunova, Peter Schaap, Johan van den Berg and Jacques Benen. Section Fungal Genomics, Laboratory of Microbiology, Wageningen University, Dreijenlaan 2, 6703 HA, The Netherlands

Contrary to the phosphorolytic degradation of D-galacturonic acid in bacteria and plants, non-phosphorolytic metabolism of D-galacturonic acid has been shown to occur in *Aspergillus* species (Uitzetter et al., 1985). This short pathway begins with the conversion of D-galacturonic acid to galactonate by an aldoketo reductase. Subsequently a dehydratase or racemase modifies galactonate to 2-keto-3-deoxygalactonate and an aldolase splits 2-keto-3-deoxygalactonate into pyruvate and glyceraldehyde. Although, *A. nidulans* mutants defective in the racemase and aldolase activities, known as *gaa*B and *gaa*A, respectively, were previously reported (Uitzetter et al., 1985), the genes encoding these activities have not been described yet. Transcriptome analysis of *Aspergillus niger* cultures grown on D-galacturonic acid, enabled the identification of a cluster of co-expressed genes that, among others, encode the necessary putative aldoketo reductase, racemase and aldolase. Sequence analysis of the genes orthologous to the *A. niger gaa*A and *gaa*B from the two *A. nidulans* D-galacturonate non utilizing mutants demonstrated the presence of point mutations in the coding regions of both *A. nidulans gaa*A and *gaa*B leading to the production of non-functional proteins and thus to the mutant phenotype. Comparative analysis of the *A. niger gaa*A and *gaa*B genes in publicly available fungal genomes revealed the presence of conserved orthologs to both genes in various distantly related fungi which suggests the presence of a common utilization pathway of D-galacturonic acid in fungi.
Reference: Uitzetter JH, Bos CJ, Visser J., *J Gen Microbiol.* 1986 132: 1167-72.

186. Location and Analysis of Syntenic Regions common to 3 Ascomycete Genomes. Diego Martinez, Michael R. Altherr. Los Alamos National Laboratory, P.O. Box 1663, Los Alamos, NM 87545.

The wealth of whole genome data now available within the ascomycete branch of fungi is able to speed the process and improve the quality of annotation. Here we utilize the close relationship of Neurospora crassa and *Aspergilus nidulans* to annotate and analyze the newly sequenced *Trichoderma reesei* genome. We aligned genetic markers from *N. crassa* and *A.nidulans* to each other and to *T. reesei* to search for syntenic regions that are conserved between two of the species or are conserved in all three of the species. Once identified these regions allow for the verification of genes based on position in the syntenic regions and other non-coding elements in the identified syntenic blocks to be verified by position as well as the standard amino-acid similarity. The creation of a database that include such large conserved regions will enrich our understanding of how genomes evolve or change over time, and will increase our knowledge of regions and motifs involved in regulation of gene transcription.

187. Withdrawn

188. A Genomic Assessment Of Novel Multi-functional Protein Sequences In The Soybean Pathogen *Phytophthora sojae*. Austin, Ryan [1], Nicholas Provart[1], and Paul F. Morris [2] [1]Department of Botany, University of Toronto, Toronto, Ont. CANADA; [2]Biological Sciences, Bowling Green State University, Bowling Green OH, USA.

The close arrangement of genes in clusters in the *Phytophthora sojae* genome http://genome.jgi-psf.org/index.html may have facilitated a trend towards greater numbers of multifunctional proteins than in other eukaryotic organisms. For example, in the shikimate pathway leading to the synthesis of aromatic amino acids, we have identified four novel multifunctional proteins in addition to the pentafunctional enzyme (ARO1) that is also present in fungi. Multifunctional proteins in the oomycetes are not limited to metabolic pathways. They also include proteins involved in nuclear trafficking, signal transduction and membrane proteins. Novel multifunctional proteins are of interest for several reasons. A higher proportion of these genes may yield identifiable phenotypes by gene silencing. Multifuctional enzymes in key biosynthetic pathways are also a genetic resource that could be tapped to increase the nutritional quality of our foods. To identify the complete set of multifunctional proteins in the P. sojae genome, the predicted transcripts were downloaded from the DOE-JGI website. The sequences were subjected to BLASTx analysis against the NR database and protein motifs were identified by InterProScan software. The output was parsed into an SQL database on the Botany Beowolf Cluster. Candidate genes with multiple motifs, where the best BLASTx hit aligns to a fraction of the total length of the proteins are then selected for visual inspection on the DOE-JGI browser. Visual inspection is being used to evaluate models for inclusion in this data set. A partial survey has so far identified more than 50 such proteins.

189. Microtubule dynamics during hyphal growth and branching in *Neurospora crassa*. Rosa R. Mouriño-Pérez[1], Robert W. Roberson[2] and Salomon Bartnicki-García[1]. [1]Departamento de Microbiología. Centro de Investigación Científica y Educación Superior de Ensenada. Ensenada, B. C. Mexico. [2] School of Life Sciences. Arizona State University. Tempe, AZ. USA.

Neurospora crassa (N2524) with GFP-tagged microtubules (MTs) is an excellent strain to study dynamics of the microtubular cytoskeleton. By confocal microscopy, we analyzed MT behavior during hyphal growth and branching. Images were assembled in space and time for a precise picture of the 3-D organization of the microtubular cytoskeleton and a clear view of its dynamics. Cytoplasmic MTs are mainly arranged longitudinally along the hyphal tube. Straight segments are rare; most MTs show a distinct helical curvature with a long pitch and a tendency to intertwine with one another to form a loosely braided network throughout the cytoplasm. This study revealed that the microtubular cytoskeleton of a hypha advances as a unit: as the cell elongates, i.e., it moves forward by bulk flow. Nuclei appeared trapped in the microtubular network and were carried forward in unison as the hypha elongated. During branching, one or more MTs became associated with the incipient branch. Branch MTs are continuous with a set of adjacent MTs from the parent hypha. Originally transverse, MT orientation turned longitudinal as the branch elongated and the length and number of MTs increased. Although the exact origin of branch MTs remains an open question, the recorded evidence indicates both bulk insertion of parent-hypha MTs as well as de novo extension by anterograde polymerization. The latter conclusion was supported by FRAP studies showing evidence of MT assembly in the growing tip of the developing branch. Nuclei entered the branch entrapped in the advancing network of MTs.

190. Secondary metabolite biosynthetic gene clusters in filamentous fungi. William C. Nierman[1], Natalie Fedorova[1], Catherine M. Ronning[1], Resham Kulkarni[1], David Denning[2], Michael Anderson[2], Masayuki Machida[3], Katsuhiko Kitamoto[4], Kiyoshi Asai[5], Joan Bennett[6], Gary Payne[7], Jiujiang Yu[8], Deepak Bhatnagar[8], Thomas E. Cleveland[8], Jennifer Wortman[1], and Jacques Ravel[1]. [1]The Institute for Genomic Research, Rockville, MD 20850, USA [2]School of Medicine and Faculty of Life Sciences, The University of Manchester, Stopford Building, Manchester M13 9PT, UK, [3]Research Center for Glycoscience, National Institute of Advanced Industrial Science and Technology , Tsukuba, Ibaraki, 305-8566, Japan. [4]Department of Biotechnology, University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan [5]National Institute of Advanced Industrial Science and Technology, Tokyo, Japan. [6]Tulane University, New Orleans, LA, USA [7]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA [8]USDA/ARS, Southern Regional Research Center, 1100 Robert E. Lee Boulevard, New Orleans, Louisiana 70124 USA

Aspergillus fumigatus pathogenicity and competition for resources may be augmented by its numerous secondary metabolites. Secondary metabolite biosynthetic genes are typically organized in clusters containing most if not all of the structural enzyme genes required for product biosynthesis. The *A. fumigatus* genome contains 24 clusters with polyketide synthase, non-ribosomal peptide synthase or dimethylallyl tryptophan synthase genes which range in size from 20 kb to 60 kb and contain from 6 to 22 genes. These clusters are dispersed throughout the genome with only 8 of the 24 located in subtelomeric regions. Many *A. fumigatus* clusters contain regulatory genes, genes associated with resistance to the metabolite, and apparently unrelated genes with no obvious role in production of the metabolite in question. Twelve clusters contain genes encoding transcription factors involved in the regulation of polyketide production and other secondary metabolite biosynthetic pathways.
With the availability of several other sequenced *Aspergilli* and related filamentous fungi, we have undertaken a comparative analysis of the secondary metabolite biosynthetic genes and clusters. Some *A. fumigatus* clusters have orthologs in *Aspergillus nidulans* or *Aspergillus oryzae* while most are *A. fumigatus* specific. We will report on these and other comparative aspects of these clusters, including chromosomal localizations, cross-species cluster integrity, inter- and intra- species relatedness of paralogous genes and the likelihood of interspecific and perhaps interkingdom horizontal gene transfer.

191.Withdrawn

192.Withdrawn

193. Comparative genomics and cross-species microarray hybridization with *Sordaria macrospora* and *Neurospora crassa*. Minou Nowrousian[1], Carol Ringelberg[2], Jennifer J. Loros[2], Jay C. Dunlap[2], Christian Würtz[1], Stefanie Pöggeler[1], Ulrich Kück[1]. [1]Lehrstuhl f. Allgemeine u. Molekulare Botanik, Ruhr-Universität Bochum, 44780 Bochum, Germany; [2]Dartmouth Medical School, Departments of Genetics and Biochemistry, Hanover, NH 03755, USA

Comparative genomics is a powerful tool to identify functional regions within genomes. It requires sequence information from closely related organisms. Here, we present a comparison of 85 genes from *Sordaria macrospora* to their *Neurospora crassa* orthologues as well as a comparison of several regions of genomic DNA containing up to five genes (Nowrousian et al. Fungal Genet Biol 41: 285-292). These data show that *S. macrospora* and *N. crassa* share a high degree of sequence identity and that the genomes of the two species are highly syntenic. Thus, *S. macrospora* sequence information can be used to simplify the annotation of the *N. crassa* genome. Furthermore, *N. crassa* cDNA microarrays can be hybridized with *S. macrospora* targets. With this cross-species array hybridization, we have identified genes that are more than twofold up- or downregulated in three developmental mutants of *S. macrospora*. Among the genes that are up- or downregulated in the sterile mutant strains are the pheromone precursor genes, genes involved in cell wall biosynthesis, and putative regulatory genes. Gene expression analysis in double mutants using Northern blots and quantitative real time PCR indicates that several regulatory pathways form a complex network controlling gene expression during fruiting body development.

194. Proteome analysis of secreted proteins from *Aspergillus oryzae* in submerged and solid-state culture conditions. Ken Oda, Dararat Kakizono, Osamu Yamada, Haruyuki Iefuji, Osamu Akita, Kazuhiro Iwashita. National Research Institute of Brewing, 3-7-1, Kagamiyama, Higashihiroshima, Hiroshima, 739-0046, Japan

Filamentous fungi are widely used for the production of homogeneous and heterogeneous proteins. *Aspergillus oryzae* is focused on because of its ability of production of heterogeneous proteins in solid-state culture. To clarify whole overview of protein secretion by *A.oryzae* in solid-state culture, we carried out comparative proteome analysis of secreted proteins in solid-state culture and submerged culture. Secreted proteins, which were prepared from both culture for 0, 12, 24, 32, and 40hr, were subjected to 2-D electrophoresis, and protein spots of 40hr were identified by peptide mass finger printing using MALDI-TOF MS. Cell-wall bound proteins of 40hr submerged culture were also identified. One hundred twelve from solid-state and 110 spots from submerged culture were analyzed. From them, 82 spots were positively identified derived from 29 gene products in total. The protein secretion profile was revealed and the identified proteins were classified into 4 groups by secretion pattern. Northern analyses of 7 genes (TAA, bgl1, glaA, glaB, xynG1, xynG2, dppV) from these 4 groups were carried out to examine the mechanism that control solid-state specific protein production. These data suggested that *A. oryzae* altered the manner of protein secretion at posttranscriptional and transcriptional level with respond to culture condition.

195. Gene expression and functional analysis during spore germination and appressorium formation of *Magnaporthe grisea*. Y. Oh, N. Donofrio, H. Pan, T. Mitchell, R. A. Dean. Fungal Genomics Lab, North Carolina State University, Raleigh NC.

Magnaporthe grisea, the casual agent of rice blast disease, develops a specialized infection structure called appressorium to gain access into its host. To identify the genes uniquely expressed during spore germination and appressorium formation, we performed microarray experiments using the *M. grisea* 22K element oligonucleotide chip created in collaboration with Agilent Technology. The microarray contains 13,666 *M. grisea* and 7,124 rice elements. RNA was extracted from harvested spores as well as from spores incubated for 7 and 12 hours on either appressorium-inductive (hydrophobic) or non-inductive (hydrophilic) surfaces. At 7 hours of incubation on the inductive surface, the tip of the germ tube had started to swell and form an initial appressorium. Fully melanized appressoria were evident at 12 hours. Spores incubated on the non-inductive surface elaborated long germ tubes but no appressoria. Expression data was analyzed using the commercial software package Genespring and verified by reverse transcription PCR. Genes significantly up regulated during appressorium formation were subjected to functional characterization by targeted gene deletion. The results of gene expression studies and gene knock-out experiments will be presented.

196. *Aspergillus flavus* genome sequence: initial analysis. G. A. Payne, B.L. Pritchard, J. Yu, W.C. Nierman, R.A. Dean, D Bhatnagar and T.E. Cleveland.

Aspergillus flavus is a widely distributed filamentous fungus that normally occurs as a saprophyte in the soil or on decaying organic matter. It is pathogenic to plants, insects and animals and produces aflatoxin, one of the most toxic and carcinogenic naturally occurring compounds. *A. flavus* is also the second leading cause of aspergillosis in humans. A whole genome sequencing project funded by the USDA/NRI and USDA/ARS and conducted at TIGR is nearing completion. Preliminary studies of the 33.1 Mb draft sequence, which consists of 17 scaffolds representing 2995 contigs, indicate that the genome contains approximately 13,800 genes. Thus the *A. flavus* genome appears to be larger than either *A. nidulans* or *A. fumigatus*. Aspergillus species are of interest in part because of the large range of clinically and industrially important secondary metabolites they synthesize. An initial scan of the A. flavus genome for proteins involved in the production of these secondary metabolites revealed the presence of 24 putative polyketide synthases and 25 putative non-ribosomal peptide synthases. Manual annotation and analysis of the genome is being coordinated through North Carolina State University and will be made available at www.Aspergillusflavus.org.

197. Functional Genomics of early interactions ectomycorrhizal symbiosis: Study of Ras mediated signaling pathways. Podila, G.K. Dept. of Biological Sciences, University of Alabama in Huntsville, Huntsville, AL 35899, USA

The formation and maintenance of functional ectomycorrhiza requires dynamic signaling interactions between the plant host and the fungus, leading to recognition between the partners and establishment of a symbiotic organ. However, the signaling pathways involved in mycorrhizal symbiosis are not well understood. We have cloned a symbiosis-regulated gene, Lbras, from the ectomycorrhizal fungus *Laccaria bicolor*, which has been shown to be associated with signaling pathways controlling cell differentiation, proliferation and apoptosis in multicellular organisms. Functional similarity of Lbras to other known eukaryotic ras proteins was established by complementation in yeast and transformation of mammalian cells. Further analysis Ras-mediated signaling pathways in *L. bicolor* during symbiosis was studied through protein-protein interaction using yeast two-hybrid system. We have identified several interaction partners for Lbras including *L. bicolor* PF6.2, previously isolated and characterized in our laboratory. We have also identified Lbras interacting small hsp class proteins and vesicular transport proteins. These results will enable us to understand the ras-interacting partner(s) of the symbiosis-related pathway(s) and contribute towards understanding the signaling pathways involved in various stages of development of ectomycorrhizal symbiosis. The recent progress made in *L. bicolor* genome sequence analysis and its implications for deciphering signaling pathways will also be discussed.

198. Structure and dynamics of *Magnaporthe grisea* telomeres. Cathryn Rehmeyer[1], Motoaki Kusaba[2], Weixi Li[1], Chuck Staben[1], Bruce Birren[3], and Mark Farman[1]. [1]Department of Plant Pathology, University of Kentucky, Lexington, KY USA. [2]Faculty of Agriculture, Saga University, Saga, Japan. [3]The Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA USA.

In many eukaryotic microbes, telomere regions harbor genes with important roles in niche exploitation. Frequent recombination in these locations is believed to produce the variation that fuels adaptation. Some *Magnaporthe grisea* telomeres are known to harbor avirulence genes, suggesting that localization of genes to chromosome ends may contribute to pathogenic adaptation in this fungus. To test this hypothesis, we have sequenced approximately 40 kilobases from each telomere in *M. grisea*. We present a summary of genes identified at chromosome ends, focusing on those which may have roles in pathogenicity. Extensive duplications and exchanges are apparent among multiple chromosome ends, and may be a possible mechanism of variation for genes that reside in these regions. The characterization of sequence exchanges among ends, as well as the sequencing of a *de novo* telomere formed during culture, are providing further insights into the mechanisms of telomere hypervariability. In addition, we have identified tandem and dispersed repeats unique to subtelomeric regions and variable in copy number among ends. Of the 14 telomeres, 11 contain ordered arrays of short conserved repeats in subtelomeric regions, the composition and organization of which suggests a functional significance. Telomere-linked RecQ helicase genes are found exclusively between subtelomeric repeat arrays and the terminal repeats; we further characterize the members of this previously described gene family. Aside from yeast, this represents the first full characterization of a complete set of chromosome ends from an organism.

199. Absence of free iron induces the expression of non-ribosomal peptide synthetases in Aspergillus fumigatus. Reiber, Kathrin, Reeves, Emer P., Neville, Claire M., Winkler, Robert*, Gebhardt, Peter*, Kavanagh, Kevin and Doyle, Sean. National University of Ireland Maynooth

The filamentous fungus Aspergillus fumigatus is a human pathogen, causing devastating, invasive diseases mainly in the respiratory system of the immunocompromised hosts. Apart from an array of mycotoxins, contributing to its aggressive appearance, the fungus produces siderophores which are responsible for iron accumulation. Siderophore production appears to contribute to organismal virulence and it is becoming clear that non-ribosomal peptide synthetases (NRPS) are involved in hydroxamate siderophore biosynthesis. We report here the characterisation and analysis of two NRPS encoding genes, termed pesB and pesF. RT-PCR analysis confirms that expression of both pesB and F is up-regulated under conditions of iron limitation concomitant with increased siderophore production. In addition, a protein corresponding to the predicted molecular mass of pesB (~200 kDa) has been identified by MALDI TOF peptide mass fingerprinting and MALDI LIFT TOF/TOF MS peptide sequencing following SDS-PAGE and 2D-PAGE separation of protein extracts obtained from mycelia grown under iron-limiting conditions. Proteomic characterisation of other high molecular mass proteins is ongoing to enable identification of other siderophore synthetases in Aspergillus fumigatus. Because siderophore synthetases are unique in bacteria and fungi they display an attractive target for anti-fungal chemotherapy to reduce the virulence of Aspergillus fumigatus.

200. Functional Genomics and Proteomics of *Trichoderma* Antagonist Strains for Industry and Agriculture. Rey Barrera, M*., Llobell, A. and Monte, E.. *Newbiotechnic, S.A. (NBT) Parque Industrial de Bollullos A-49 (PIBO). 41110 Bollullos de la Mitación. Sevilla. Spain.

The genome of the *Trichoderma* antagonists is poorly surveyed compared with other model microorganisms, due to the great diversity of its species, the absence of optimised systems for its exploration, and the great variety of genes expressed under a wide range of ambient conditions. The EU-project (QLK3-2002-02032) "TrichoEST" started under the 5th Framework Programme "Quality of Life" December 1. 2002. The aims are to identify genes and gene products from *Trichoderma* spp. with biotechnology value, to assess their industrial potential and, to exploit and commercialize them in concert with EU biotechnology strategy. The project is not a sequencing programme. Instead, it develops integrated functional genomic and proteomic approaches and innovative use of bioinformatics, leading to rapid exploitation of genes and gene products and their transformation into industrial processes. Technologies achieved will have applications in a range of agro-industrial, environmental and medical activities, including innovations in agricultural pest and pathogen management, novel antibiotics, and enzymes with industrial uses involving processing of plant-derived matter, including animal feed, composting and bioremediation. The project is coordinated by Dr. Manuel Rey Barrera, Newbiotechnic S.A., Spain, and the partners, besides us, are Dr. Fabrice Lefevre, Proteus S.A., France, Dr. Antonio Llobell González, University of Seville, Spain, Dr. Enrique Monte Vázquez, University of Salamanca, Spain, Dr. Matteo Lorito, University of Napoli, Italy, Dr. Christian Kubicek, Technical University of Vienna, Austria, Dr. Gabi Krczal, Centrum Grüne Gentechnic, and Dr. Paul Cannon, CAB International, UK.

201. Identification of genes in the *Gibberella zeae* sexual reproductive pathway by cross genome comparison. Barbara Robbertse and Gillian Turgeon Department of Plant Pathology, Cornell University Ithaca, NY 14853

The sexual development pathway of *Saccharomyces cerevisiae* (*Sc*) is almost certainly the best-understood fungal developmental pathway. How conserved is this pathway in filamentous fungi, specifically in *Gibberella zeae* (*Gz*)? Large-scale functional analysis of Sc, large-scale expression analysis of *S. pombe* (*Sp*), and perithecium-specific EST sequences of *Neurospora crassa* (*Nc*), *Magnaporthe grisea* (*Mg*), and *Gz* are available to study this question. We downloaded the predicted protein sequences of *Sc*, *Sp*, *Gz*, *Nc*, and *Mg* and performed blastp searches combined with Tribe-MCl and INPARANOID analyses to find all possible orthologs among the protein sequences of these fungi. In total, 3,034 putative *Sc* orthologs and 3,311 putative *Sp* orthologs were identified in *Gz*. Four large-scale functional studies and the SGD database have identified a non-redundant set of 610 genes as essential for *Sc* sexual development. Thus, the candidate *Gz* ortholog data set was filtered for all *Sc* genes with a mating/meiosis/sporulation-specific phenotype when deleted and for all *Sp* genes induced during mating, meiosis or sporulation. Approximately 44% of the 610 essential *Sc* genes have orthologs in *Gz*. Of these, 47 *Gz* genes were identified that had matches to perithecial ESTs. Fifty two percent (528) of induced *Sp* genes were orthologous to *Gz* and 82 of these *Gz* orthologs had matches to perithecium specific ESTs. The 47 *Sc*-*Gz* orthologs and 82 *Sp*-*Gz* orthologs had only 7 *Gz* orthologs in common. Deletion studies are underway to confirm that the *Gz* orthologs, identified by cross genome comparison, are essential for sexual development.

202. Defining the Secretome of *Fusarium graminearum*. John S. Scott-Craig, Kohhei Otani, Frances Trail$, Heather E. Hallen$, Brett Phinney*, Janet M. Paper, Neil Adhikari, and Jonathan D. Walton. MSU-DOE Plant Research Laboratory, *Genome Technology Support Facility, $Department of Plant Biology, Michigan State University, East Lansing, Mi 48824, USA

We are using mass spectrometric-based proteomics to identify the secreted proteins of the plant pathogenic fungus *Fusarium graminearum*. Proteins and peptides are being identified from culture filtrates and mycelial wall extracts of *F. graminearum* grown on eight different media. The range of carbon sources used includes purified maize and carrot cell walls, pectin, xylan, casein, collagen, dried milk and sucrose. Intercellular wash fluids from *F. graminearum*-infected wheat heads are also being extracted and analyzed. By combining the publicly available, high-quality sequence of the *F. graminearum* genome with state-of-the-art instrumentation for proteomics, an analysis of the "secretome" (that is, the totality of secreted proteins) of a plant pathogenic fungus is now possible. These results complement and extend past biochemical, genetic, and genomic studies on secreted fungal proteins and their regulation. The results will contribute novel and useful information of interest to fungal biologists and especially to plant pathologists. In particular, the work should lead to the identification of proteins or peptides that contribute to the interaction between *F. graminearum* and its host.

203. Conservation of synteny among filamentous fungi. Michael R. Thon[1] and Ralph A. Dean[2]. [1]Program for the Biology of Filamentous Fungi, Department of Plant Pathology and Microbiology,Texas A&M University, College Station, TX 77843, USA. [2]Center for Integrated Fungal Research, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA We performed pairwise comparisons between *Magnaporthe grisea* chromosome VII and the genome sequences of *Fusarium graminearum*, *Aspergillus nidulans*, and *Neurospora crassa* to identify regions of conserved synteny among the fungi. Segmental homologs (i.e. syntenic blocks) containing 5 or more genes ($P < 0.001$) were identified using the algorithm and statistical test implemented in the FISH software package. 21 blocks were found in N. crassa, 17 in *F. graminearum*, and 2 in *A. nidulans*. In general, the blocks were roughly co-linear and interspersed with intervening, non-syntenic genes. The syntenic blocks were restricted to *N. crassa* chromosome I, *F. graminearum* chromosome II and IV, and *A. nidulans* chromosome III, suggesting that these chromosomes share common ancestry and/or a preference for intrachromosomal rearrangements. A segment of conserved synteny was found that spans all four species that is 50-75 genes in length, of which eight genes are conserved in all four species and 14 genes are conserved in three out of four species. We will present a summary of conservation of synteny between *M. grisea* chromosome VII and the genomes of related fungi, and discuss the implications for the evolution of fungal genomes.

204. Nutrient sensors in *Candida albicans*: role of Gpr1 in response to glucose limitation and amino acid deprivation. Hélène Tournu, Mykola Maydan, Larissa de Rop, Joke Serneels, Patrick Van Dijck Flanders Interuniversity Institute for Biotechnology, Department of Molecular Microbiology, VIB10, Laboratory of Molecular Cell Biology, K.U. Leuven, Kasteelpark Arenberg 31, B-3001 Leuven, Belgium

We and others have recently shown that the G-protein-coupled receptor Gpr1 is involved in the morphogenic transition between yeast and hyphae in the human pathogen *Candida albicans*. Phenotypic analyses on hyphal-inducing media such as the amino acids based medium Lee showed a clear defect in filamentous growth in a strain deleted for GPR1. Because of the nature of that particular medium, we investigated further the putative ligand of CaGpr1. Although glucose has been shown to be the ligand of *Saccharomyces cerevisiae* Gpr1, a strain lacking a functional Gpr1 in *C. albicans* is still capable of inducing a transient cAMP increase in response to the addition of glucose. Amongst the seven amino acids included in Lee's medium, methionine is essential to the wild type hyphal growth, but addition of methionine does not restore filamentation in a gpr1 null. Could CaGpr1 have an amino acid as a ligand? Preliminary results show that addition of methionine to cells growing in presence of limited amount of glucose induces an increase of cAMP in wild type but not in gpr1 null. Addition of a methionine analogue does not induce filamentation, and picomole amounts of L-methionine are sufficient to trigger the morphogenic switch in wild type cells suggesting a possible sensing mechanism.

205. Divergence in gene expression related to variation in host specificity of an ectomycorrhizal fungus. Antoine Le Quéré[1], Andres Schützendübel, Balaji Rajashekar[1], Björn Canbäck[1], Jenny Hedh[1], Susanne Erland[1], Tomas Johansson [1]and Anders Tunlid[1]. [1] Department of Microbial Ecology, Lund University, Lund, Sweden; [2] Present address: Department of Forest Botany and Tree Physiology, Georg-August-University, Göttingen, Germany.

There is a considerable variation in the degree of host specificity among species and strains of ectomycorrhizal fungi. In this study, we have for the first time shown that this variation is associated with quantitative differences in gene expression, and with divergence in nucleotide sequences of symbiosis regulated genes. Gene expression and sequence evolution were compared in different strains of the ectomycorhizal fungus *Paxillus involutus* including Nau, that is not compatible with birch and poplar, and the two compatible strains Maj and ATCC200175. On genomic level, Nau and Maj were very similar. In contrast, 66 out of the 1,075 genes were differentially expressed in Maj compared to Nau after contact with birch roots. Thirty-seven of these symbiosis-regulated genes were also differentially expressed in the ATCC strain. Comparative analysis of DNA sequences of the symbiosis-regulated genes in different strains showed that two of them have evolved at an enhanced rate in Nau. The sequence divergence can be explained by a decreased selection pressure, which in turn is determined by lower functional constraints on these proteins in Nau as compared to the compatible strains.

206. Genome sequences of *Phytophthora sojae* and *P. ramorum* shed new light on their evolution and pathogenicity. Brett M. Tyler[1], Sucheta Tripathy[1], Igor Grigoriev[2], Daniel Rokhsar[2,3], Jeffrey Boore[2,3] and many others. [1]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg VA 24061; [2]DOE Joint Genome Institute, Walnut Creek, CA; [3] University of California, Berkeley.

The approximately 60 species of *Phytophthora* are all destructive pathogens, causing rots of roots, stems, leaves and fruits of a huge range of plants important to agriculture, horticulture and natural ecosystems. These pathogens are not fungi but belong to the oomycete group of the kingdom Stramenopiles. We have determined draft DNA sequences of the 95 Mb genome of the soybean pathogen *Phytophthora sojae* and of the 65 Mb genome of the sudden oak death pathogen *Phytophthora ramorum*, to a depth of 9x and 7x respectively. The genome sequences show clearly for the first time that oomycetes, which are heterotrophic, have evolved from a photosynthetic ancestor, which they share with photosynthetic Stramenopiles such as diatoms. The *Phytophthora* genomes also contain several bacterial genes not present in any other eukaryotes. The genomes show rapid diversification of proteins associated with plant infection such as hydrolases, ABC transporters, protein toxins, proteinase inhibitors and avirulence gene products, but relatively little diversification of biosynthetic genes for metabolite toxins. The *P. sojae* and *P. ramorum* genomes show substantial synteny (colinearity) except in regions encoding putative pathogenicity genes. The genome sequences have also enabled new tools such as an Affymetrix™ gene expression microarray for *P. sojae* together with its host soybean, and an Affymetrix™ SNP chip for tracking *P. ramorum* spread.
Supported by USDA grant 2002-35600-12747 and NSF grant MCB-0242131 to B. Tyler and DOE Genomes to Life funding to the DOE JGI.

207. The interplay of the pectinase spectrum of *Aspergillus niger* as revealed by DNA microarray studies. Elena Martens, Peter Schaap, Johan van den Berg and Jacques Benen. Fungal Genomics group, laboratory of Microbiology, dept. AgroTechnology and Food Sciences, Wageningen University, Dreijenlaan 2, 6703 HA Wageningen.

The saprophytic fungus *Aspergillus niger* is an efficient producer of extracellular enzymes. Previous research has revealed that many of these enzymes show carbohydrate modifying activities. These enzymes are Generally Regarded As Safe (GRAS status) and are therefore widely used in the food industry.
Recently DSM Food Specialties solved the genomic sequence of this fungus. Based on the new data, it was estimated that only a fraction of the potential of enzymes secreted by *A. niger* is currently characterized. Database mining using the proprietary genome sequence has resulted in the identification of at least total thirty-eight genes encoding enzymes involved in the depolymerisation of the backbone of the complex polysaccharide pectin. Additional enzymatic activities are required to degrade the arabinogalactan side chains attached to the main chain and to remove methyl and acetyl esters that are present in this main chain.
By applying the powerful technology of DNA microarrays we have sought to gain insight into the complex regulation of the expression of all the genes involved in pectin degradation. For this we have cultivated *A. niger* on sugar beet pectin and on the monomeric sugar constituents of pectin, viz galacturonic acid, rhamnose and xylose, and subsequently analysed the corresponding transcriptomes using microarrays. We will report on our findings concerning the regulation of the expression of the genes involved in the degradation of pectin and the consequences for the interplay of the encoded (novel) enzymes.

208. Differential gene expression by *Metarhizium anisopliae* growing in root exudate and host (*Manduca sexta*) cuticle or hemolymph reveals mechanisms of physiological adaptation. Chengshu Wang, Gang Hu and Raymond J. St. Leger. Department of Entomology, University of Maryland, 4112 Plant Sciences Building, College Park, MD 20742, USA.

Like many other fungal pathogens *Metarhizium anisopliae* is a facultative saprophyte with both soil-dwelling and insect pathogenic life-stages. In addition, as *M. anisopliae* traverses the cuticle and enters the hemolymph it must adapt to several different host environments. This implies considerable physiological plasticity. In this study we used expressed sequence tags (ESTs) and cDNA microarray analyses to demonstrate that physiological adaptation by *M. anisopliae* to insect cuticle, insect hemolymph, bean root exudate (a model for life in the soil) and nutrient rich Sabouraud dextrose broth (SDB) involves different subsets of genes. Overall, expression patterns in cuticle and hemolymph clustered separately from expression patterns in root exudates and SDB, indicative of critical differences in transcriptional control during pathogenic and saprophytic growth. However, there were differences in gene expression between hemolymph and cuticle and these mostly involved perception mechanisms, carbon metabolism, proteolysis, cell surface properties and synthesis of toxic metabolites. Some of these differences were of clear adaptive significance. Thus, cuticle-degrading proteases were switched off during growth in hemolymph, avoiding activation of the host prophenoloxidase system. In other cases differences suggested previously unsuspected stratagems of fungal pathogenicity that can be tested experimentally. Examples include large-scale changes in gene expression of cell wall proteins in hemolymph, as these may alter interactions with host defense responses. The results of this study will initiate a detailed molecular analysis of the ecological traits that adapt an ascomycete to live both in the soil and as a pathogen of insects.

209. The Evolutionary Genomics of 5S rRNA Genes in Filamentous Fungi: Patterns of Multigene Family Evolution and Gene Amplification. Todd J. Ward and Alejandro P. Rooney. Microbial Genomics & Bioprocessing Research Unit, National Center for Agricultural Utilization Research, USDA Agricultural Research Service, Peoria, IL 61604

The 5S gene is the smallest of the four nuclear ribosomal RNA (rRNA) genes in eukaryotes. In some species the gene is part of the regular rRNA tandem array, while in others the gene is dispersed. The 5S rRNA genes of filamentous fungi form a large multigene family of 50 to 100 copies. During the course of our studies on the genomic evolution of filamentous fungi, we found that these genes display varying degrees of divergence from one another, suggesting that they do not evolve in a concerted manner. This pattern is quite unusual because nuclear rRNA genes represent a paradigm of concerted evolution. The purpose of this study was to characterize the patterns of multigene family evolution and evolutionary genomics of 5S rRNA genes in the genomes of representative filamentous fungi. Our studies show that the genes undergo birth-and-death evolution under strong purifying selection. Furthermore, the rate of gene turnover appears to be quite high in these genomes. Because the 5S genes in these species are dispersed across the genome, our findings indicate that the mechanisms controlling the multiplication and movement of 5S genes across the genome are highly dynamic. As such it appears that a process resembling retroposition controls 5S rRNA gene amplification, dispersal and integration in the genomes of filamentous fungi.

210. Proteomic Identification And Functional Analysis of Extra-Cellular Proteins from *Magnaporthe grisea*. SHENG-CHENG WU, Kumar Kolli, Erica Berelc, Evan Conroy, Peter Albersheim, Alan Darvill, Ron Orlando. Complex Carbohydrate Center, University of Georgia, Athens, GA 30602

Magnaporthe grisea, the rice blast fungus, secretes copious amount of extracellular proteins (ECPs) in response to various growth conditions. These ECPs are believed to play indispensable roles in diverse biological activities such as nutrition uptake, growth and cell-cell communication and molecular interactions between the pathogen and its host. We have used high-throughput proteomics technologies such as multidimensional liquid chromatography-mass spectrometry (MDLC/MS) to identify a number of *M. grisea* ECPs. Although about half of the ECPs are putatively associated with a known function such as glycoside hydrolysis, the others are novel, with a few being "infection growth-specific". Gene knockout and necrosis/alkalinization analyses are being carried out to evaluate some ECP's role in pathogenicity and fungus-plant interactions, and tandem affinity purification (TAP) will assist with identifying host proteins possibly interacting with the ECPs. *[Supported by U.S. Department of Energy grants DE-FG05-93ER20221 and DE-FG02-93ER20097, the National Science Foundation grant NSF-9626835 and the National Institutes of Health grant P41RRR05351]*

211. Draft sequence of Monascus sp. BCRC 38072 genome. Gwo-Fang Yuan, Chun-Lin Wang, Chien-Chi Chen, Li-Ling Liaw, Wen-Shen. Chu, Shih-Hau Chiu, Jyh-Wei Chen, Tzu-Pei Chiu, Li-Ming Sung, Ing-Er Hwang, Hsiao-Chi Peng, Hui-Wen Chang, Mei-Chih Chien, Yi-Ju Liu and Chii-Cherng Liao,. Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, ROC

The filamentous fungus Monascus sp. can produce various secondary metabolites useful as food additives and/or pharmaceuticals. A Monascus strain, BCRC 38072, isolated from red rice can produce monacolin K, a cholesterol serum synthesis inhibitor. The genome sequence of Monascus sp. BCRC 38072 was obtained by whole-genome shotgun of a variety of clone types (plasmid, fosmid and BAC) at 11-fold sequence coverage. The Arachne package was used to assemble the genome sequence. A total of 673,853 highly qualified reads were input into the assembly program. The resulting draft consists of 709 contigs, larger than 2 kb in length, with the total length of 26.8 Mb. Seventeen major supercontigs were assembled covering 94.8% of the whole assembly length and 422 ungrouped contigs were assembled in the rest 5.2%. The N50 lengths of supercontig and contig are 2.5 Mb and 224 Kb, respectively. A total of 6,326 genes were predicted with 6,226 encoding proteins longer than 100 amino acids. Among the predicted proteins, 1,739 (27.5%) and 2,312 (36.6%) have significant matches to the known Saccharomyces cerevisiae proteins and proteins from SwissProt, respectively.

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212. Withdrawn

213. Identification and transcriptional regulation of new starch modifying enzymes in the *Aspergillus niger* genome. X. Yuan[1], R. Van der Kaaij[2], E. Martens[3], C.A.M.J.J. van den Hondel[1,4], and A.F.J. Ram[1,4]. [1] Leiden University, Institute of Biology Leiden, Fungal Genetics Research Group, Leiden, The Netherlands. [2]University of Groningen, Microbial Physiology Research Group, Haren, The Netherlands. [3] Wageningen University, Section Fungal Genomics, Wageningen, The Netherlands. [4] TNO-Nutrition and Food Research, Department of Microbiology, Zeist, The Netherlands.

Aspergillus niger is well known for its ability to secrete a wide variety of plant Carbohydrate Modifying Enzymes (CMEs). The secreted CMEs are able to degrade the plant cell wall materials as well as its storage carbohydrates, like starch. Based on its recently finished genomic sequence by Dutch life sciences company DSM, it is estimated that only a fraction of the potential of enzymes produced by *A. niger* is currently exploited.
Database mining of the *A.niger* genome resulted in the identification of twelve new enzymes, which contain motifs indicating that they might act on starch. In addition to the known starch degrading enzymes of *A.niger*, glucoamylase (*glaA*), two alpha-amylase (*amyA* and *amyB*), an acid amylase (*aamA*) and an alpha-glucosidase (*aglU*), six putative alpha-amylases, five putative alpha-glucosidases and one alpha1,4-glucan branching enzyme were identified. It is well established that the expression of starch modifying enzymes is coordinately regulated at the transcriptional level by a pathway specific transcription factor AmyR. AmyR binds to sequences (CGGN $_8$ CGG or CGGN $_8$AGG) in the promoter region of amylolytic genes. Inspection of the promoter regions of the newly identified amylolytic genes revealed that only five of them contain AmyR consensus binding sites. The expression of the newly identified genes in relation to the presence of starch and the role of AmyR during regulation has been examined using microarrays and will be presented.

214. The Fungal Hydrophobin RolA Bound on Hydrophobic Surfaces Recruits Esterase. Toru Takahashi[1], Hiroshi Maeda[2], Sachiyo Yoneda[1], Shinsaku Ohtaki[1], Yohei Yamagata[1,2], Fumihiko Hasegawa[2], Katsuya Gomi[1,2], Tasuku Nakajima[1,2], and Keietsu Abe[1,2,*], [1]Grad. Sch, of Agricul. Science, [2]The New Indust. Creat.Hatchery Cent., Tohoku Univ., Sendai, Japan

When fungi grow on plant or insect surfaces coated with wax polyesters as protectants against pathogens, the fungi generally form aerial hyphae to contact the surfaces. Hydrophobins, which are surface-active proteins found in fungi, coat aerial structures such as hyphae or conidiophores and are involved in adhesion to hydrophobic surfaces. When an industrial fungus *Aspergillus oryzae* was cultivated in liquid medium containing the biodegradable polyester polybutylene succinate-coadipate (PBSA), the hydrophobin RolA and cutinase CutL1, which hydrolyzes PBSA, were simultaneously expressed. High levels of RolA and its localization on the cell surface in the presence of PBSA were confirmed by immunostaining. Preincubation of PBSA with RolA stimulated PBSA degradation by CutL1, suggesting that RolA bound to a PBSA surface was required for the stimulation. Immunostaining revealed that PBSA films coated with RolA specifically adsorbed CutL1. Quartz crystal microbalance analyses further demonstrated that RolA attached to a hydrophobic sensor chip specifically adsorbed CutL1. These results suggest that RolA adsorbed to the hydrophobic surface of PBSA recruits CutL1, resulting in condensation of CutL1 on the PBSA surface and consequent stimulation of PBSA hydrolysis.

215. Heterologous Protein Expression in a Filamentous Fungus. Silke Alves and Frank Kempken. Abteilung für botanische Genetik und Molekularbiologie, Botanisches Institut und Botanischer Garten, Christian-Albrechts-Universität Kiel, Am Botanischen Garten 1-9, 24118 Kiel, Germany

In the last years filamentous fungi have become more important in expression of biotechnologically used proteins. They are capable to secrete a broad range of different proteins into the supernatant of the culture medium. Their ability to perform post-transcriptional modifications makes them an attractive host for the production of heterologous proteins. However, the amount of heterologous proteins rarely exceed more than a few milligrams per litre, due to problems during the secretion process and extracellular protease activity of the fungi. In this study, we constructed a set of different expression-vectors that enable us to exchange different genes, promoters and terminators without difficulties. To investigate the applicability of these expression-system, we tested the expression of eGFP and several other genes in filamentous fungi. Each expressed gene includes a specific fungal export-signal, which enables the secretion of the processed protein into the supernatant. Different promoters are being employed and tested under various physiological conditions.

216. Directed evolution of barley beta-D-glucan endohydrolases. Graham Eariss [1], Maria Hrmova [2], Geoffrey Fincher [2] & David E. A. Catcheside[1]. [1]School of Biological Sciences, Flinders University, Adelaide, South Australia. [2]Department of Plant Science, University of Adelaide, South Australia.

We are using an *in vivo* gene diversification technique developed in *Neurospora crassa* to investigate the functional divergence of a barley (1-3) and (1-3,1-4)-beta-D-glucanase, and to attempt to produce a (1-3,1-4)-beta-D-glucanase with enhanced thermostability. The primary sequences and three dimensional structures of the barley (1-3)-beta-D-glucan endohydrolase isozyme GII and (1-3,1-4)-beta-D-glucan endohydrolase isozyme EII imply a close relationship in evolutionary terms, yet they perform completely different functions. While the (1-3)-beta-D-glucanase capable of hydrolyzing the linear, substituted and branched (1-3)-beta-D-glucans often found in fungal cell walls appears to be involved in plant protection, the (1-3,1-4)-beta-D-glucanase is responsible for digestion of the starchy endosperm cell wall during germination of barley grains. Possibly in response to the hostile environment it encounters, the (1-3)-beta-D-glucanase has evolved to be significantly more thermostable than the (1-3,1-4)-beta-D-glucanase.

217. Multiplex PCR for the detection of ochratoxigenic fungi in coffee beans. Maria Helena Pelegrinelli Fungaro*; Daniele Sartori, Márcia Cristina Furlaneto.Centro de Ciências Biológicas, Universidade Estadual de Londrina, P.O.Box, 6001, 86051-990 Londrina-PR, Brazil.

Ochratoxin A (OA) is a nephrotoxic and carcinogenic mycotoxin that has been found in cereal and food commodities. This mycotoxin was originally described as a secondary metabolite of *Aspergillus ochraceus* strains, but in subsequent years other *Aspergillus* and *Penicillium* species were described as OA producers. Nowadays, *Aspergillus carbonarius*, *A. niger* and *A. ochraceus* have been recognized as culprit species of OA in coffee beans. This situation demonstrates the importance of using appropriate methods to control the mycological status of coffee beans. The use of DNA markers for easy and rapid detection of these species in the interior of coffee beans can be useful in order to substitute the conventional methods, which are based in fungal cultivation and requires an expertise in taxonomy. In this study, strains representing closely related black aspergilli species were analyzed by molecular markers aiming at the development of specific primers for detection of *A. niger* in coffee beans. The primer pair designed in this study and other two primers pair developed for *A. carbonarius* and *A. ochraceus* were used in the multiplex-PCR optimization. Our group has successfully used this method in order to assess the presence of these ochratoxigenic species in coffee beans samples.
Acknowledgement CAPES/CNPQ/Fundação Araucária/CPG-UEL

218. Expression and characterization of xylanases from *Aspergillus fumigatus*. Haiyan Ge, Janine Lin, Suzie Otani and Debbie S. Yaver. Novozymes Biotech, Inc, Davis CA.

Xylanase, which catalyzes the endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans, are used in the food industry, primarily for baking and in fruit and vegetable processing such as fruit juice production or wine making. Using bioinformatics, three putative GH10 xylanase genes, *xyn1,xyn 2,* and *xyn3*, were discovered in *A. fumigatus* genome. The alignment showed that the deduced amino acid sequence of *xyn1* shared 72% identity to NCU08189.1 of the *Neurospora crassa*; the deduced amino acid sequence of *xyn2* shared 54% identity to XynF1 of the *Aspergillus oryzae*, and the deduced amino acid sequence of *xyn3* shared 67.8% identity to Xynia of the *A. aculeatus*. We successfully cloned and expressed the three putative xylanases in *A. oryzae*. Xylanase activities were assayed using AZCL-arabinoxylan as substrate. On SDS-PAGE gel, proteins with expected sizes bands were observed. Further characterization of the xylanses will be presented.

219. Directed Evolution of Human Growth Factors in *Neurospora crassa*. Steven T. Henderson[1,2], Leah J. Cosgrove[2], Colin W. Ward[3] & David E. A. Catcheside[1]. [1]Flinders University, Adelaide, SA 5042, Australia; [2]CSIRO Division of Health Sciences and Nutrition, Adelaide, SA 5000, Australia; [3]CSIRO Division of Health Sciences and Nutrition, Parkville, VIC 3052, Australia.

This project aims to generate novel human growth factors (hGF) by utilising the *in vivo* gene diversification system developed for the filamentous fungus, *Neurospora crassa*. Directed evolution of hGFs in Neurospora will utilise the high frequency of meiotic recombination initiated at the cog^L recombination hotspot to diversify hGF DNA sequences juxtaposed to cog^L during a sexual cross. Specifically, targeting vectors will be used to transplace a functional hGF gene between *his-3* and cog^L. Repeat-Induced Point mutation (RIP) is a natural phenomena occurring in the pre-meiotic phase of Neurospora's sexual stage that results in G:C to A:T transition mutations in duplicated sequences. Thus, an additional non-functional hGF gene will be ectopically transformed into Neurospora to induce low frequencies of RIP, generating hGF alleles *in vivo*. Additional hGF variation is created by subsequent meiotic recombination shuffling the hGF alleles created by RIP. This has the additional advantage of potentially separating deleterious mutations. Progeny from the cross will be screened to identify novel hGF variants.

220. Aspergillus nidulans uvsC and genetic transformation. Yasuo Itoh. Shinshu University, Nagano, Japan

Gene targeting to knock out the activity of specific genes has become important due to recent progress in genomics research. But this technique is still unavailable for many organisms, including economically important microorganisms, due to the high background of ectopic integration during genetic transformation. *Aspergillus nidulans uvsC* is an ortholog of budding yeast *RAD51* that plays central roles in recombination-based repair and meiotic recombination. Occurrence of gene targeting at *argB* locus was under a detection limit in a null mutant of *uvsC* (less than 5% of control strains) when randomly chosen transformants were examined for their integration profiles. Other differences were also observed in the integration profiles during genetic transformation that were consistent with the expected functions of *uvsC*. Transcription of *uvsC* was elevated using the 5• f sequences of the glyceraldehyde-3-phosphate dehydrogenase (P*gpd*) and Taka-amylase A (P*taa*) genes from *A. nidulans* and *A. oryzea*, respectively. Maximum of 5-fold increase in the efficiency of targeting was observed at *argB, yA, and wA* loci when *uvsC* was under control of P*gpd*. Higher level of transcription was achieved with P*taa*, but at the inducible condition with maltose, mycelial growth was significantly suppressed. These results demonstrate that *uvsC* is involved in integration of extra-cellular DNA integration into chromosomes and is a possible rate-limiting factor for gene targeting. However the increased efficiency of gene targeting is hindered by a deleterious effect of increased transcription on cell proliferation.

221. Targeted disruption of fungal genes based on a dual selection method. Chang Hyun Khang[1], Sook-Young Park[1], Hee-Sool Rho[2], Yong-Hwan Lee[2], and Seogchan Kang[1]. [1]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, USA; [2]School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea

The creation of gene knock-out (KO) mutants through homologous recombination is the most direct method to determine gene function. In filamentous fungi, however, this method is often hampered by the low efficiency of homologous recombination, which requires molecular analysis for a large number of transformants to identify gene KO mutants. We have developed an efficient gene KO method, which is based on *Agrobacterium tumefaciens*-mediated transformation with a binary vector containing a mutant allele of the target gene flanked by a negative selection marker, the herpes simplex virus thymidine kinase (*tk*) gene. Ectopic transformants are eliminated through a negative selection resulting from the expression of the *tk* gene, thus significantly enriching for gene KO mutants. The efficiency of gene KO by this method was evaluated using *Magnaporthe grisea* and *Fusarium oxysporum*. Although a significant enrichment of gene KO mutants was achieved in both fungi, false positives occurred mainly due to deletion of the *tk* gene on the T-DNA. An array of gene KO vectors were developed and tested for their efficiency in gene KO.

222. Overexpression of basidiomycetous enzymes in *Coprinopsis cinerea*. S. Kilaru, M. Rühl*, A. Saathoff, R. C. Dwivedi, M. Zomorrodi, K. Lange, A. Majcherczyk, P. Hoegger and U. Kües. Georg-August-University of Göttingen, Institute of Forest Botany and NHN*, 37077 Göttingen, Germany

Basidiomycetous fungi are the source of many biotechnologically interesting enzymes, e.g. laccases, peroxidases, and others. When such enzymes are attempted to be overexpressed in ascomycetous fungi, they often show low yields and have altered properties, most probably due to improper posttranslational modifications. A commonly observed problem is the overglycosylation of the proteins. Therefore, we developed an efficient expression system in *Coprinopsis cinerea* as a basidiomycetous host. The laccase gene *lcc1* of *C. cinerea* served as a tool to establish the best activities among various homologous and heterologous promoters. Enzymes produced under control of foreign promoters exhibit natural extents of glycosylation. Various laccases with different activities and specificities have now been obtained from recombinant *C. cinerea*. With our expression system, we were able to produce much higher amounts of enzyme than obtained from natural inductive promoters in this and other white rot fungi. The purification, biochemical characterization and comparison of the different overexpressed laccases is now in process.
Our laboratory is supported by the Deutsche Bundesstiftung Umwelt (DBU), the NHN by the European Regional Development Fund and the country of Lower Saxony.

223. Characterization of the telomere-attached vectors and their transformants of *Aspergillus oryzae*. Ken-Ichi Kusumoto, Ikuyo Furukawa, Tae Kimura, Mayumi Matsushita, Satoshi Suzuki, and Yutaka Kashiwagi (National Food Research Institute, JAPAN)

The authors isolated the telomere sequences at the chromosomal terminal regions of *Aspergillus oryzae*, and showed that their structure was a repeated sequence constituted of 12bp repeat unit (TTAGGGTCAACA) (AMB61: 247, 2003). As telomere is participated in the replication of the chromosomal DNA and its stabilization of their ends, development of new type of the replicating vectors is expected. The replicating function of the telomeres of *A. oryzae* and the conservation of the chromosomal ends by them are not investigated yet. In this study, we constructed the two types of the telomere-attached vectors to examine their function. The vector I was constituted of single copy of TEL134, a clone of *A. oryzae* telomere $(TTAGGGTCAACA)_{10}$ with its associated sequence, inserted into a vector of a integrated type, pPTRI (ptrA as a marker gene, TaKaRa Bio, JAPAN). The vector II was constituted of two copies of TEL134 inserted into pPTRI, where the two TEL134 were inserted as inverted repeat. A 0.8 kb DNA fragment derived from a vector for *Escherichia coli* was put as a spacer sequence between those TEL134 DNA. Namely, the spacer sequence was positioned as vector-$(TTAGGGTCAACA)_{10}$-spacer-$(TGTTGACCCTAA)_{10}$-vector. *A. oryzae* NFRI1599 were transformed with those telomere-attached vectors and pPTRI as a control plasmid. We are going to characterize the structure of the introduced vectors and the transformants.

224. Withdrawn

225. A transfer of key primary metabolism genes of *Aspergillus niger* into *Aspergillus terreus*. G. Tevž[1], M. Benèina[1], A. Kern[2], A. Glieder[2], M. Legiša[1]. [1]National Institute of Chemistry, Hajdrihova 19, Si-1001 Ljubljana, Slovenia. [2]Institute for Molecular Biotechnology, Technische Universitaet Graz, A-8010, Graz, Austria

In *Aspergillus niger*, one of the most important industrial micro-organisms, strong anaplerotic reactions were found to be responsible for high productivity of primary metabolites. Two enzymes, 6-phosphofructo-1-kinase (PFK), a key regulatory enzyme of glycolytic pathway, and alternative oxidase, a membrane protein that uncouples NADH re-oxidation from ATP formation, have been recognised to play a key role in replenishing citric acid cycle intermediates. After a transfer of genes coding for both enzymes into *Aspergillus terreus* cells, a similar effect on the level of tricarboxylyc acid cycle intermediates was expected to be achieved and concomitantly increased productivity and/or yield of specific *A. terreus* bio-products. The genes *pfk*A coding for 6-phosphofructo-1-kinase and *aox*1 carrying the information for synthesis of alternative oxidase were isolated from *A. niger* gene library by the aid of the known sequence. Namely, both genes have been previously isolated, cloned and sequenced by other laboratories. The genes together with their native promoter and terminator regions have been integrated into appropriate vectors. Heterologous *pfk*A gene was introduced into *A. terreus* protoplasts by co-transformation with a plasmid carrying *hyg*B gene as a marker for hygromycine resistance, while double transformants were isolated after additional co-insertion of a plasmids carrying *aox*1 and *amd*S gene. The integration of genes was confirmed by standard recombinant DNA techniques. In cell free extract of the modified strain higher specific activity of PFK was detected by comparing to the parental strain and significantly higher sensitivity of double transformant was recorded toward SHAM, a specific inhibitor of alternative oxidase. The obtained results indicate that *A. niger* genes could be efficiently expressed in other *Aspergillus* species, while their effect on primary metabolism will have to await further studies.

226. Purification, characterization, and expression of AP025 protease from *Thermoascus aurantiacus*. Janine Lin, Wenping Wu, Jan Lehmbeck, and Debbie Yaver. Novozymes Biotech, Inc, Davis CA.

A metalloprotease AP025 was purified from culture broth of a thermophilic fungus *Thermoascus aurantiacus*. The AP025 protease has high optimal temperature (70˚C), and is most active at low pH (pH 6). The enzyme is most stable at pH 4, but is also stable from pH 4-10. The AP025 protease may have potential in industrial applications. The gene encoding the protease AP25 was cloned and sequence analysis showed that this protease is a metalloprotease. The cDNA of the AP025 gene was successfully expressed under two variants of *Aspergillus niger* amylase promoter in *Aspergillus oryzae* and in *Fusarium venenatum* under the control of the *F. venenatum* glucoamylase promoter. Expression of AP025 under a stronger promoter in *A. oryzae* resulted in the highest yield of AP025.

227. Genetic stability of direct and inverted chromosomal repeats in *Aspergillus nidulans.* Michael Lynge Nielsen, Line Albertsen & Uffe Mortensen, Center for Microbial Biotechnology, BioCentrum-DTU, DK-2800 Kgs. Lyngby, Denmark

Homologous recombination facilitates error free repair of DNA double strand breaks because it uses a homologous sequence as a template for the repair. However, most genomes contain repetitive sequences such as transposable elements, rDNA, centromeric repeats, etc, which in some cases cause inadvertent homologous recombination leading to chromosomal translocations or genomic instabilities. In many filamentous fungi, including *Aspergillus nidulans*, transformation of recombinant DNA often leads to the integration of multiple copies of this DNA either as inverted or tandem repeats. For many industrial applications transformants with multiple gene copies are obtained from screening because such strains often have high gene expression levels, but consequently, the repeated sequences result in genetic instability. For filamentous fungi there are no accurate methods for assessing this type of genetic instability. Therefore, we have developed a sensitive conidiospore color assay in *A. nidulans* that allows the detection of as little as a single spontaneous or induced recombination event within a fungal colony and also determines whether the event proceeded as a loop-out or a gene conversion event. The assay provides a unique way to measure the recombinogenic effects of specific direct or inverted repeats, and can effectively be used to measure the genotoxicity of compounds or the effect of various gene mutations on recombination.

228. Genetic modification of carbon catabolite repression in *Trichoderma reesei* for improved protein production. Tiina Nakari-Setälä[1], Marja Paloheimo[2], Jarno Kallio[2], Jari Vehmaanperä[2], Merja Penttilä[1], and Markku Saloheimo[1]. [1]VTT Biotechnology, Espoo, Finland and [2]Roal Oy, Rajamäki, Finland.

The *cre1* gene mediates the carbon catabolite repression in *Trichoderma reesei* in the presence of glucose. The CREI protein has been shown to bind to specific sequences in the promoter of the major cellulase gene *cbh1*. CREI target sequences have also been identified in the promoter regions of other cellulase and hemicellulase genes such as *cbh2* and *xyn1*. A truncated form of CREI (CREI-I), present in the hypercellulolytic *T. reesei* strain Rut-C30, has been shown to be responsible for derepression of cellulase and hemicellulase gene expression on glucose-containing media. We modified the carbon catabolite repression of *T. reesei* by deleting the wild type *cre1* gene from the *T. reesei* strains QM6a and VTT-D-80133, a hypercellulolytic mutant from the strain QM9414. In addition, QM6a transformants were constructed in which the *cre1* gene was replaced by the “Rut-C30 -type” truncated *cre1-1* gene. Bioreactor cultivations were carried out with both types of *cre1* mutant strains. Increases in the levels of protein and cellulase production were detected compared to the parents, both when the strains were grown on glucose and on lactose based complex medium. Higher levels of increase compared to parent were detected from the QM6a transformants. The results obtained from the cultivations and the expression analysis will be described.

229. Fungal biosensors based on novel luminous and fluorescent probes. Emma Perfect and Patrick Hickey. LUX Biotechnology

Luminescent and fluorescent proteins form an increasingly important component of the biologist's tool kit. These non-invasive probes are proving to be invaluable for investigations into the dynamics and localisation of cellular components and have aided drug discovery programmes, the development of biosensors and helped understand plant-fungal interactions.
In addition to being a distributor of fluorescent and luminescent genes, proteins and reagents, LUX is using its expertise in fungal molecular biology and imaging to develop fungal biosensors for environmental testing, drug discovery and R+D. These biosensors harness the power of bioluminescence and fluorescence to generate strains whose light output allow a monitoring of fungal health as well as indicating the presence of specific molecules in the environment. The characteristics of a variety of LUX biosensors, which incorporate a range of fluorescent, luminescent and calcium regulated photoproteins are presented.

230. Proteolysis and protein processing in filamentous fungi. Peter J. Punt, Margreet Heerikhuisen, Cora M.J. van Zeijl, B. Christien Lokman and Cees A. M. J. J. van den Hondel. *TNO Quality of Life, Department of Microbiology, P.O.Box 360, 3700 AJ Zeist, The Netherlands*
Filamentous fungi are being as production organisms for fungal and, more recently, non-fungal proteins. Our research is focused on three different areas of research related to efficient protein production:**(i)** protease mutants,**(ii)** protein processing in the secretion pathway,**(iii)** vacuolar proteases
(i). Already early on, protease production was addressed as a bottleneck in achieving high levels of secreted protein. Several approaches to isolate strains with reduced protease production, including a new direct selection approach and options for functional genomics and HTS-type of approaches will be discussed
(ii). A "secretion carrier" approach results in increased levels of secreted heterologous protein. To analyze the role of the KEX2/furin like protein processing protease in this approach, we have isolated the corresponiding fungal *kexB/pclA* genes and analyzed the effect of gene-disruption on protein secretion. The resulting mutant strains show significantly increased levels of several fusion proteins. Detailed analysis of some of the secreted fusion proteins revealed the presence of alternative, PclA- independent, protein processing pathways.
(iii) . Also protein targeting to the vacuole and release of vacuolar proteases is identified as possible reasons for low levels of secreted proteins. To study this we have isolated vacuolar protease mutant strains to study proteolysis and protein processing in vacuolar protease mutants.

231. *ThPTR2* , A PEPTIDE TRANSPORTER GENE FROM *T. harzianum* CECT 2413. Vizcaíno, J. A[1]., Cardoza, R. E[2]., Hermosa, R[1]., Rey-Barrera, M[3]., Gutiérrez, S[2]. and Monte, E[1]. [1]Centro Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca. Edificio Departamental. Lab 208. Plaza Doctores de la Reina s/n. 37007. Salamanca, Spain. [2]Microbiology Area. University of León. Campus of Ponferrada. Ctra. Astorga s/n, 24400. Ponferrada. 987-442000 Fax. 987-442070. [3]Newbiotechnic, S.A. (NBT) Parque Industrial de Bollullos A-49 (PIBO). 41110 Bollullos de la Mitación. Sevilla. Spain.

Some *Trichoderma* strains are used as biological control agents (BCAs) of important plant-pathogenic fungi. Due to the interest of *Trichoderma* species, a functional genomic project ("TrichoEST") was undertaken by an International Consortium comprised of academic institutions and enterprises. The aims were to identify genes and gene products from *Trichoderma* spp. with biotechnology value.
The PTR (peptide transport) family transporters are found in both prokaryotes and eukaryotes (fungi, plants and animals). Members of this family have been shown to transport a wide range of nitrogen-containing substrates, including amino acids, peptides and nitrate [1]. *PTR2* from *S. cerevisiae* has been the only fungal PTR gene studied so far [2].
We decided to study the EST 6935, obtained from an EST library that was made from conditions related to biocontrol, due to its homology to PTR genes. Here, we report the cloning and characterization of the gene *ThPTR2*. This gene was 1,738 bp long and contained one 64-bp intron. *In silico* study of the codified protein (557 aa) predicted a 12 transmembrane domain transporter as expected. A *Northern* analysis of *ThPTR2* expression was performed and at last, using a protoplast-mediated transformation strategy, we overexpressed *ThPTR2* in *T. harzianum* CECT 2413 (homologous) and *T. longibrachiatum* T52 (heterelogous expression).
[1] Stacey, G., Koh, S., Granger, C. and Becker, J. M. (2002) Trends Plant Sci, 7, 257-263.
[2] Perry, J. R., Basrai, M. A., Steiner, H. Y., Naider, F. and Becker, J. M. (1994) Mol Cell Biol, 14, 104-115.

232. Withdrawn.

233. Characterization of the chitinolytic system of *Trichoderma*: development of a novel screening method to monitor gene expression on multiple carbon sources. V. Seidl, B. Huemer, B. Seiboth, I. Druzininha and C.P. Kubicek

The mycoparasitic fungus *Trichoderma atroviride* antagonizes the growth of several plant pathogenic fungi. Different hydrolytic enzymes such as chitinases and glucanases are involved in the cell wall degradation of the host. Biomining of the sequenced genome of *T. reesei*, which is industrially used to produce cellulolytic and hemicellulolytic enzymes, revealed a total of 19 genes encoding putative chitinases. 11 of these genes encode novel, so far not characterized chitinases. Based on these chitinase genes from *T. reesei*, 5 new chitinase encoding genes from *T. atroviride* P1 were isolated and sequenced. Origin and diversity of the novel chitinases from *T. atroviride* were asessed by phylogenetic analysis. Transcription of the 5 new chitinases differs from previously characterized chitinases: only low basal transcription levels, but no induction upon growth on *Rhizoctonia* cell walls and chitin were found. In order to identify novel, potent inducers of chitinase gene expression, a new method based on phenotype microarrays (containing 96 different carbon sources), which were developed for studying the global carbon utilization pattern of *Trichoderma* strains, was applied. Expression patterns of exo- and endochitinases of the wild-type and Gox-reporter strains of the two major chitinases of *T. atroviride* were monitored on a variety of carbon sources. The data show that phenotype microarrays in combination with reporter strains could be a useful tool to characterize the expression of novel enzymes.

234. REMI mutagenesis and identification of infection defective mutants in Wheat Scab Fungus *Fusarium graminearum*. Kye-Yong Seong[1], Jiqiang Yao[1], H.Corby. Kistler[2], Jin-Rong Xu[1]. [1]Dept of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907. [2]USDA-ARS, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Fusarium graminearum is an important pathogen of small grains and maize in many areas of the world. Wheat scab (head blight), caused primarily by *F. graminearum* in North America, poses a major threat to wheat production. To better understand the molecular mechanisms of plant infection and virulence of F. graminearum, we used the REMI (Restriction-Enzyme Mediated Integration) approach to generate random insertional mutants. Eleven pathogenicity mutants were identified by screening 6,500 hygromycin-resistant transformants. In mutant M8, the transforming plasmid was integrated 110-bp upstream from the start codon of the cystathionine beta-lyase gene (CBL1). Gene replacement mutants deleted for CBL1 was also obtained. The cbl1 mutants were methionine autotrophic and significantly reduced in virulence, indicating that the methionine synthesis pathway is important for pathogenesis in F. graminearum. We also have identified genes disrupted by the transforming DNA in three other REMI mutants. In mutants M68, the transforming vectors were inserted in the NADH: ubiquinone oxidoreductase. The putative b-ZIP transcription factor gene ZIF1 and the transducin beta-subunit-like gene TBL1 disrupted in mutants M7 and M75, respectively, had no known homologs in filamentous fungi and were likely to be novel fungal virulence factors. Further characterization of ZIF1 and TBL1 genes are under the way.

235. Efficient degradation of dichlorodibenzo-*p*-dioxins by recombinant basidiomycete *Coriolus hirsutus* strains. K. Shishido[1], K. Orihara[1], H. Kawashima[1], Y. Okajima[1], T. Yamazaki[1], T. Sakaki[2], A. Tsukamoto[3] and J. Sugiura[3]. [1)]Department of Life Science, Tokyo Institute of Technology, Yokohama, Japan, [2)]Biotechnology Research Center, Toyama Prefectural University, Toyama, Japan, [3)]Advanced Technology Research Laboratory, Oji Paper Co. Ltd., Shinonome, Tokyo, Japan.

Basidiomycete extracellular lignin-degrading enzymes catalyze degradation of polychlorodibenzo-*p*-dioxins (PCDDs). The plasmid MIp30-lip containing the expression cassette of *C. hirsutus* lignin peroxidase (LiP) gene (*lip*) was introduced into protoplasts of *C. hirsutus* monokaryotic strain OJ1078, obtaining two good transformants ChTF3-1(Ch.LiP) and ChTF3-2(Ch.LiP) which carry several copies of the *lip* expression cassette on their chromosomes. Their culture supernatants showed the 2,7-DCDD-degrading activities 2-3 times higher than control strain. Rat cytochrome P450, CYP1A1 plays an important role in metabolism of PCDDs. The plasmid MIp5-(cyp1a1+arg1) containing the rat CYP1A1-expression cassette was introduced into protoplasts of OJ1078, obtaining three good transformants ChTF5-2(CYP1A1), ChTF5-4(CYP1A1), and ChTF5-6(CYP1A1) which carry 9, 6, and 7 copies of the expression cassette on their chromosomes respectively. Their microsomal fractions contained similar amounts of rat CYP1A1. The three transformants degraded 2,7/2,8(1:1)-DCDDs six times more efficiently than OJ1078.

236. Characterization of a Fungal Gene Provides Technologies for Transgenic Bioremediation and Biosensing of Environmental Pollutants. Kristin M. Skinner and Lynda M. Ciuffetti, Department of Botany and Plant Pathology, Oregon State University

Graphium, a filamentous ascomycete, grows on *n*-alkanes. In *Graphium*, these unlikely growth substrates are rendered more palatable through an initial oxidation reaction, which produces the corresponding alcohol. The alcohol is shuttled through conventional metabolic pathways to provide energy and carbon for growth. After exposure to alkanes, *Graphium* cultures cometabolically oxidize a broad range of xenobiotic compounds including fuel oxygenates, chlorinated solvents, and aromatic hydrocarbons. Because these compounds do not support growth, subsurface conditions and biological limitations may impede *Graphium's* ability to metabolize xenobiotics *in situ*. However, transfer of the metabolic capabilities of the fungus into a different organism may result in new technologies for the remediation of polluted groundwater. Biochemical characterization of alkane metabolism in *Graphium* will facilitate this technology. Competition and inhibition assays suggest that both direct and cometabolic oxidation activity are catalyzed by an inducible cytochrome P-450 alkane monooxygenase (Alk1). A putative gene encoding Alk1, *Grsp Alk1*, has been identified and cloned. Although sequence analysis and expression data suggest that *Grsp Alk1* encodes Alk1, heterologous expression and targeted gene disruption will ultimately assign Alk1 function. The inducible promoter region directly upstream of the putative *Grsp Alk1* gene was also characterized. Four upstream regions were cloned and used to drive expression of Green Fluorescent Protein (GFP) in *Graphium* and in *Verticillium dahliae*. Fluorescence was quantified in the presence of different substrates to determine if low concentrations of xenobiotics are sufficient to drive GFP expression. These results may lead to the development of strains that can directly measure the presence or absence of toxic compounds in environmental samples.

237. Expression of fish antibodies in the filamentous fungus *Trichoderma reesei*. Valentino S. Jr. Te'o [1,2], Gemma C. MacDonald [1], Peter L. Bergquist [1,2,3], Stewart Nuttall [4], Helena K.M. Nevalainen [1,2]. [1] Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia. [2] Macquarie University Biotechnology Research Institute, Sydney, NSW 2109, Australia. [3] Department of Molecular Medicine & Pathology, Auckland University Medical School, Auckland, New Zealand. [4] CSIRO Health Sciences and Nutrition, 343 Royal Parade, Parkville, Victoria, Australia

The increasing interest on recombinant low volume-high value products of pharmaceutical importance, typically of mammalian origin, has strengthened the attraction in using filamentous fungi as expression hosts. For example, the shark immunoglobulin new antigen receptor (IgNAR) antibodies which possess single variable domains (V_{NAR}), lack the external hydrophobic region present in traditional antibodies (1). These small V_{NAR} molecules have enormous potential in therapeutics because they can penetrate dense tissues to access unique epitopes (1).
Functional and correctly folded shark IgNAR variable domain (V_{NAR}) antibodies have been produced in *E. coli* at levels of 3-5 milligrams per litre. We present here our results from the expression of the hydrophilic IgNAR molecules (V_{NAR} 12F-11 and 14M-15) in the protease deficient *T. reesei* strain RutC30, using different fusions to the highly secreted CBHI protein.
(1) Nuttall SD, Krishnan UV, Hattarki M, De Gori R, Irving RA, Hudson PJ (2001). Isolation of the new antigen receptor from wobbegong sharks, and use as a scaffold for the display of protein loop libraries. Mol Immunol 38 : 313-326.

238. Genetic transformation of *Ascochyta rabiei* using Agrobacterium-mediated transformation. David White, Tony Chen, and Weidong Chen. Agriculture Research Service/Washington State Univ.

The conditions for efficient transformation of *Ascochyta rabiei* using *Agrobacterium tumefaciens*-Mediated Transformation produce random integration events have been determined. Conidiaspores of an *A. rabiei* pathotype II isolate were co-cultivated with *A. tumefaciens* carrying a virulence plasmid to determine the efficiency of T-DNA insertion into the fungal genome. Pre- and co-cultivation conditions were varied to determine the optimal transfer efficiency. Randomness and frequency of T-DNA integration were measured and related to co-cultivation conditions. T-DNA insertions were stably maintained in the absence of selective pressure and during asexual development. The efficiency of T-DNA transfer of a hygromycin resistance cassette driven by the plant-derived CaMV35s promoter was found to be less efficient than an identical T-DNA molecule carrying an *Aspergillus nidulans* trpC promoter driving expression of hph. Increased co-cultivation times of *A. tumefaciens* cells with *A. rabiei* conidiaspores resulted in an increased rate of transformation into the *A. rabiei* genome without a significant increase in the number of insertions per genome. The growth phenotype of transformants was media-dependent in the presence of Hygromycin. A subset of *A. rabiei* transformants were screened for the loss of pathogenicity on chickpea cultivars using a minidome assay. Two transformants unable to infect chickpea were isolated for further analysis.

Host Parasite Interactions

239. Employing Polymorphic Gene Sequences as Tool for Investigating the Epidemiology of *Trichophyton tonsurans* Infection. Susan Abdel-Rahman, Andrea Gaedigk. Division of Pediatric Clinical Pharmacology, Children's Mercy Hospital. Kansas City, MO 64108.

Despite the fact that dermatophytoses represent the first human disease attributed to a microorganism, the dermatophyte genomes remain largely uncharacterized and little information is available exploring fungal carriage and infection on a molecular level. Using three gene loci, we have developed a genotyping algorithm which affords the ability to discriminate strains of *T. tonsurans*. All sequence variations were stable upon serial passage over 18 months suggesting that these loci provide a sufficiently sensitive means for longitudinal genotyping. With this approach, we have undertaken a large scale longitudinal epidemiologic study designed to 1) describe the genetic relatedness of strains that cause active infection vs. carriage and 2) determine whether the carrier state that has been previously described represents transient acquisition of different genetic strains or persistent colonization with a unique strain. Cultures are collected monthly from over 150 children attending an urban daycare center with over 12 months of data accumulated to date.
On average, 43% of individuals are positive every month for fungal growth consistent with *T. tonsurans*. A sufficient quantity of high quality DNA is available to confirm species and discriminate *T. tonsurans* strain type in 33% of the population with over 20 distinct strain types observed. Of interest, only 9% of the children sampled display signs or symptoms consistent with infection, thus illustrating the large number of fungal carriers in the pediatric population. More than 90% of the children who are culture positive on serial sampling display persistent carriage with the same strain(s). Although not wholly specific, unique sequence variations appear to segregate with strain types causing carriage versus infection.

240. Is a homolog of a host-selective toxin from *Pyrenophora tritici-repentis* at work in the *Pyrenophora bromi*-bromegrass interaction? Rachael M. Andrie and Dr. Lynda M. Ciuffetti. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR.

Pyrenophora bromi is the causal agent of brown leaf spot of bromegrass (*Bromus inermis*), a widely cultivated hay and pasture grass. The presence of conspicuous chlorotic halos surrounding brown leaf spots suggests the involvement of a phytotoxin in disease. *P. tritici-repentis*, the species of *Pyrenophora* most closely related to *P. bromi*, requires the production of multiple host-selective toxins (HSTs) to cause the disease tan spot of wheat, including the proteinaceous HSTs Ptr ToxA and Ptr ToxB. Because of the relatedness of *P. bromi* and *P. tritici-repentis*, it is conceivable that *P. bromi* contains homologous sequences to *ToxA* and/or *ToxB*, the products of which may be involved in its interaction with bromegrass. Southern analysis revealed the presence of *ToxB*-like sequences in *P. bromi*. Polymerase chain reaction (PCR) was used to clone multiple *PbToxBs* from a number of *P. bromi* isolates. Comparisons of the putative open reading frames of the *PbToxB* sequences of *P. bromi* and *ToxB* of *P. tritici-repentis* clearly indicate a high level of relatedness, though at the inferred amino acid level the PbToxB proteins are more similar to each other than with the ToxB protein. At least one copy of *PbToxB* for each of three *P. bromi* isolates is transcribed in culture and *in planta* as shown by reverse transcriptase (RT)-PCR. To assess the role of PbToxBs in the interaction between *P. bromi* and bromegrass, the corresponding *PbToxB* loci were heterologously expressed in *Pichia pastoris* and the resultant PbToxB proteins infiltrated into bromegrass and wheat. Infiltration of PbToxBs into bromegrass did not result in obvious disease symptoms; however, infiltration into wheat resulted in chlorosis.

241. A regulator of G-protein signaling in *Ustilago maydis* promotes sporulation *in planta* and suppresses hyphal growth on limited nutrients. Lori G. Baker[1], Scott Gold[2], and Sarah F. Covert[3]. [1]Department of Genetics, [2]Department of Plant Pathology, [3]Warnell School of Forest Resources. The University of Georgia, Athens, GA, U.S.A.

Ustilago maydis only produces teliospores when growing within in its host plant, *Zea mays*. The signaling pathway(s) that *U. maydis* uses to switch from vegetative growth to reproductive growth *in planta* are not well characterized, but one G-alpha subunit, Gpa3, has been shown to be a negative regulator of sporulation. Based on data from other fungi, we hypothesized that *U. maydis* uses a regulator of G-protein signaling, Rgs1, to deactivate Gpa3 and thus promote sporulation *in planta*. Deletion of *rgs1* reduced *U. maydis in planta* sporulation dramatically and over-expression of *rgs1* caused filamentous growth in liquid medium. The latter phenotype partially mimics *gpa3* deletion, thus both lines of evidence suggest that Rgs1 negatively regulates Gpa3 as predicted. However, crosses between *rgs1* deletion strains and *gpa3* constitutively active (*gpa3Q206L*) mutants were less virulent than *gpa3Q206L* x *gpa3Q206L* crosses, raising the possibility that Rgs1 regulates more than one G-alpha involved in pathogenicity. We found that the individual deletions of *gpa1* and *gpa4* caused precocious sporulation *in planta*, indicating that both Gpa1 and Gpa4 are likely to be negative regulators of sporulation in *U. maydis*. Furthermore, deletion of *rgs1* induced filamentous growth on minimal medium, while deletion of *gpa1* or *gpa4* suppressed hyphal growth on low nutrients. Preliminary evidence from double deletion mutants, suggests that functional Gpa1 or Gpa4 is essential for the filamentous phenotype of the *rgs1* deletion mutant. Therefore, our emerging model is that at least three pathways suppress sporulation in *U. maydis* and that Rgs1 down-regulates this suppression on multiple fronts.

242. Wheat leaf rust, *Puccinia triticina*, ESTs and functional analysis in a surrogate system; a rust MAPK functions in *Ustilago maydis*. Guus Bakkeren, Guanggan Hu*, Rob Linning, Andrena Kamp. Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Highway 97, Summerland, BC, V0H 1Z0, Canada; *Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada

Genomics approaches to advance the study of rust fungi are beginning to bear fruit. We are developing a 40,000-read EST database, currently at 15,000 and 4,200 unigenes, representing cDNA libraries from several developmental stages of *P. triticina* including host infection. We discovered a MAPK with homology to kinases known to be involved in pathogenicity in other fungi. It is similar to the *U. maydis* MAPK, UBC3/KPP2, but has a longer N-terminal extension of 43 aa with identities to *U. maydis* KPP6, a homolog of UBC3/KPP2 with a 170 aa extension. UBC3/KPP2 is involved in mating (and subsequent pathogenic development) whereas KPP6 functions during invasive growth in corn tissue. PtMAPK, expressed from a Ustilago-specific promoter, was able to complement a *ubc3/kpp2* mutant and restore mating. It also substantially increased virulence on corn, measured as tumor formation, of a *kpp6* mutant. Moreover, this construct restored to near-full pathogenicity a *ubc3/kpp2 kpp6* non-pathogenic double mutant. Complementation with the complete PtMAPK gene indicated that the rust promoter might be recognized in *U. maydis*. Phylogenetically, these basidiomycete plant pathogens are relatively close. This is corroborated by our Pt-EST analyses in which most sequences matching fungal genes best, are from *U. maydis*. This system opens up avenues along which rust research can be advanced, currently frustrated by the lack of transformation protocols.

243. Characterization of *yps* a putative *Aspergillus nidulans* adhesin. S. Venkatramen, A.M. Dranginis and D.C. Bartelt, Dept. of Biological Sciences, St. John's University, Queens NY 11439

In a screen of an *A. nidulans* lamda cDNA library for genes encoding calcium-binding proteins, we identified a homolog of *Histoplasma capsulatum yps* -3. *H. capsulatum* is a dimorphic fungus in which only the yeast phase is pathogenic. YPS-3p is expressed on the cell surface in a yeast phase specific manner [Weaver, C.H., K.C. Sheehan and E.J. Keath, (1996) *Infect. Immun.* 64:3048-3054]. Antibodies to YPS-3p have been found in the serum of patients with Histoplasmosis but not in normal sera. The A. nidulans cDNA encodes a 118 residue protein with a predicted N terminal signal sequence, and an EGF homology calcium-binding domain. A cDNA containing the ORF and a transcription termination sequence from *S. cerevisiae* was cloned into a yeast shuttle vector pRS426 containing a PGK promoter, and URA3 selectable marker. The resulting plasmid, pYPSA, was used to transform *S. cerevisiae* Sigma1278b flo11$^-$. FLO11p, a cell surface flocculin, is required for adhesion of yeast to the extracellular matrix proteins, fibronectin, lamin, and collagen. The *S. cerevisiae* Sigma1278b flo11$^-$ is unable to bind to fibronectin coated polystyrene in a microtiter plate assay. Of the seven yeast transformants isolated, the same four consistently displayed binding to fibronectin coated wells of a microtiter plate. These data support the characterization of YPSAp as an *A. nidulans* adhesin. Supported by NIAID R15AI062801-01.

244. Mitochondrial virulence determinants in the maize pathogen *Ustilago maydis*. Kathrin Stelter, Miriam Bortfeld, Christine Vogler, Kathrin Auffarth, Regine Kahmann and Christoph W. Basse. Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Straße, 35043 Marburg, Germany

The basidiomycete fungus *Ustilago maydis* is a facultative biotrophic pathogen causing smut disease in maize plants. The *U. maydis mrb1* gene encodes a mitochondrial protein of the p32 family. Members of this family are known from different eukaryotic organisms and have diverse regulatory functions. In *U. maydis mrb1* is necessary for pathogenic development of dikaryotic hyphae originating from *a1* and *a2* strains, while solopathogenic, haploid *a1 mrb1* mutant strains are not affected in pathogenicity. Deletion analysis showed that the *a2* mating type locus genes *lga2* and *rga2* account for the loss of pathogenicity in the absence of *mrb1* with *lga2* being the major contributor. We show that the *lga2* and *rga2* products are localized in mitochondria and provide evidence for a complex involving Mrb1, Lga2 and Rga2. Conditional overexpression of *lga2* in haploid *U. maydis* cells severely affected growth, triggered mitochondrial fragmentation as well as loss of mitochondrial DNA, and compromised respiratory activity. We provide evidence that Lga2 interferes with mitochondrial fusion and that Mrb1 is required to control this activity. *lga2* represents a direct target of the bE/bW complex and thus its expression is linked to pathogenic development. To get further insight into the consequences of *lga2* expression a microarray analysis was performed. This revealed marked transcriptional alterations related to metabolism, transport and the induction of stress responses. We suggest that *U. maydis* has developed a system that allows the regulation of mitochondrial morphology under control of the mating type loci *a* and *b* and possibly serves to cope with special stress conditions during biotrophic growth.

245. Whole genome mutagenesis in *Magnaporthe grisea*, insertional mutant analysis and recovery of DNA regions flanking the insert. Melania Betts[1], Sara L. Tucker[1], Natalia Galadima[1], Lei Li[4], Yang Meng[2], Gayatri Patel[2], Nicole Donofrio[3], Jin-Rong Xu[4], Thomas Mitchell[3], Mark Farman[2], Ralph Dean[3], Marc J. Orbach[1]. [1]University of Arizona, Tucson, AZ 85721. [2]University of Kentucky, Lexington, KY 40546. [3]North Carolina State University, Raleigh, NC 27695, [4]Purdue University, West Lafayette, IN 47907.

M. grisea is an ascomycete and the causal agent of rice blast disease. Our goal is to identify the specific genes involved in all stages of the infection cycle from attachment and colonization through lesion development and sporulation. An insertional mutagenesis approach to saturate the Magnaporthe genome was chosen as the strategy to reach this goal. Over 50,000 DNA insertion lines of *M. grisea* strain 70-15 have been generated mostly through *Agrobacterium tumefaciens*-mediated transformation. The library has been screened for defects in pathogenicity, morphology, metabolism, conidiation and growth rate. Data on the characterization of strains with interesting phenotypes is currently being generated and will be presented. A second stage of the project involves the recovery of sequences flanking the sites of insertion to look at the randomness of insertion. We are also recovering sequences from mutants that have pathogenicity defects in order to idenfity novel genes involved in the infection process.

246. Resistance and pathogenicity interactions between Arabidopsis and *Hyaloperonospora parasitica*. Jim Beynon, Rebecca Allen, Laura Baxter, Peter Bittner-Eddy, Mary Coates, Sharon Hall, Julia Meitz and Anne Rehmany. Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK

The oomycetes represent a distinct group of organisms that cause diseases in plants, fish and humans. *H. parasitica* represents an excellent choice in which to study pathogenicity in these organisms as it infects the model plant Arabidopsis. We have used subtractive and map based cloning methods to clone two pathogen genes (*ATR1* and *ATR13*) that trigger a host resistance response when the plant contains specific resistance genes. Both *ATR* genes reveal very high levels of allelic diversity and suggest an "arms race" between host and pathogen. Highly variable alleles of *ATR13* are recognised by the same resistance gene allele. In contrast, different alleles of *ATR1* trigger different combinations of resistance genes. We have used naturally occurring allele variants to define regions within these pathogen gene products that are responsible for specificity in their recognition by the resistance genes.

247. Investigation of fungal morphological differentiation in apple scab infection. Bowen JK[1,2], Rees-George J[2], Hill GN[1,2], Hahn M[3], Kemen E[4], Kucheryava N[3], Templeton MD[2], Plummer KM[1,2]. [1]University of Auckland, PB 92019, Auckland, NZ. [2]HortResearch, Mt Albert Research Centre, PB 92169, Auckland, NZ. [3]University of Kaiserslautern, Postbox 3049, 67653 Kaiserslautern, Germany. [4]Konstanz University, Germany.

The hemi-biotrophic fungus *Venturia inaequalis* causes apple scab disease. The fungus grows between the cuticle and epidermal cells during its parasitic phase. It undergoes a dramatic morphological change during infection since a stroma, which resembles laterally dividing cells rather than hyphal filaments, is formed. A similar structure is formed within cellophane *in vitro*. Fungal growth reverts to tubular hyphal growth habit on emergence from the cellophane. We are using the growth of *V. inaequalis* on cellophane as a model for stroma formation during infection of apple leaves. Fungal genes up-regulated on cellophane and during infection, compared with growth on nutrient media, are being sought as these may be involved in cellular differentiation and may also be potentially important for infection. One such gene, *CIN1* has such an expression profile, supporting the hypothesis that cellophane-grown *V. inaequalis* mimics infection processes. CIN1 is predicted to be a relatively large (~460 amino acid), highly cysteine rich protein, with repetitive amino acid motifs and a predicted signal peptide, suggesting that it is located in the cell wall or secreted externally. CIN1 is being expressed in *Pichia* to determine the structure/function of the protein and to verify peptide sequence. Suppression subtractive hybridisation is being employed to construct a cDNA library enriched for cellophane-induced genes. We plan to use gene silencing in *V. inaequalis* to facilitate the functional characterisation of genes of interest.

248. Gain of fertility mutants lost their pathogenicity in *Magnaporthe grisea*. Myoung-Hwan Chi, Sook-Young Park, Soonok Kim and Yong-Hwan Lee. School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea

Magnaporthe grisea, the causal agent of rice blast, is a heterothallic filamentous ascomycete. The fungus is considered as a model organism for studying fungal pathogen-plant interactions not only due to the great economic importance involved, but also due to the molecular genetic tractabilities. Two gain of female fertility (GFF) mutants were obtained from T-DNA mutant library of a Korean field strain *M. grisea* KJ-201, a female sterile MAT1-2. Genetic crosses of two GFFs with *M. grisea* strain 70-6, a female fertile MAT1-1, formed two lines of perithecia containing viable ascospores. Backcrosses of F1 progenies with strain 70-6 confirmed gain of female fertility. Comparison of x^2 distribution at the expected 1:1 ratio indicated single gene inheritance of a marker gene, hygromycin phosphotransferase (*HPH*) in random ascospore analysis. T-DNA insertion sites in the chromosome were identified by TAIL-PCR and sequencing. Inactivation of corresponding genes was confirmed by RT-PCR analysis. Both GFF mutants lost their pathogenicity on susceptible rice cultivar, Nagdongbyeo. Functional complementation of these mutants is in progress. This research might uncover the relationship between female fertility and pathogenicity of *M. grisea*.

249. Fungal polyketide synthase is involved in cercosporin biosynthesis and fungal virulence of *Cercospora nicotianae*. Mathias Choquer, Katherine A. Dekkers, Hui-Qin Chen, Lihua Cao, and Kuang-Ren Chung. Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred, Florida, U.S.A.

Cercosporin is a photosensitizing perylenequinone toxin produced by the plant pathogenic *Cercospora* species. A polyketide synthase gene (*CTB1*) was cloned and functionally characterized to involve in cercosporin biosynthesis in *C. nicotianae*. The *CTB1* gene product contains a keto synthase (KS), an acyltransferase (AT), a thioesterase/claisen cyclase (TE/CYC), and two acyl carrier protein (ACP) domains, and has high levels of similarity to many fungal type I polyketide synthases. Targeted disruption of *CTB1* resulted in mutants completely devoid of both *CTB1* transcript and cercosporin biosynthesis. The *ctb1*-null mutants caused fewer necrotic lesions on inoculated tobacco leaves compared with wild type. Complementation of *ctb1*-null mutants with a full-length *CTB1* clone restored wild-type levels of cercosporin production as well as the ability to induce lesions on tobacco. Thus, we have conclusively demonstrated that cercosporin is synthesized via a polyketide pathway, and cercosporin is an important virulence factor in *Cercospora* spp. Chromosome walking and sequence analysis of the surrounding DNA fragments of the *CTB1* gene, suggesting that the clustering of cercosporin biosynthetic genes, similar to many fungal secondary metabolites, also occurs in *Cercospora* spp.

250. Molecular cloning and characterization of a putative phophatidyl inositol-specific phospholipase C (PLC) gene from *Cryphonectria parasitica*. Hea-Jong Chung, Ae-Young Mo, Seung-Moon Park, Moon-Sik Yang and Dae-Hyuk Kim. Division of Biological Sciences, Basic Science Research Institute, Chonbuk National University, Dukjindong 664-14, Chonju,Chonbuk 561-756, Korea

The hypovirus is known to downregulate the fungal laccase1 (*lac*1), the modulation of which is tightly governed by the inositol triphosphate (IP3) and calcium second messenger system in a virus-free strain. We cloned the gene *cplc*1 encoding a phosphatidyl inositol-specific phospholipase C (PLC), in order to better characterize the fungal gene regulation by hypovirus. Sequence analysis of the *cplc*1 gene indicated that it contained both the X and Y domains, which are the two conserved regions found in all known PLCs, with a 133 amino acid extension between the 2nd beta-strand and the alpha-helix in the X domain. In addition, the gene organization appeared to be highly similar to that of a delta type PLC. Disruption of the *cplc*1 gene resulted in slow growth and downregulation of *lac*1 expression. However, temperature sensitivity, osmosensitivity, virulence, and other hypovirulence-associated characteristics did not differ from the wild-type strain. Functional complementation of the *cplc*1-null mutant with the PLC1 gene from *S. cerevisiae* restored *lac*1 expression, which suggests that the cloned gene encodes PLC activity. The present study indicates that the *cplc*1 gene is required for proper mycelial growth and that it regulates the *lac*1 expression, which is also modulated by the hypovirus.

251. Molecular cloning and characterization of a gene, *lac*3, encoding an inducible laccase from *Cryphonectria parasitica*. Hea-Jong Chung, Eun-Sil Choi, Min-Jae Kim, Seung-Moon Park, Tae-Ho Kwon and Dae-Hyuk Kim. Division of Biological Sciences, Basic Science Research Institute, Chonbuk National University, Dukjindong 664-14, Jeonju, Chonbuk 561-756, Korea

Although the laccase activity has shown a correlation to the hypovirulence and has been considered as one of major virulence factors, molecular characterization of its biological function has been hampered due to the functional redundancy. At least three laccases have been suggested in *Cryphonectria parasitica*: one intracellular and two extracellular forms. We cloned a novel laccase gene *lac*3. The deduced amino acid sequence appeared to contain a putative leader peptide of 18 amino acids long and characteristic four copper binding regions. Northern blot analysis of the *lac*3 gene revealed that the *lac*3 was specifically induced by tannic acid, which is abundant in the bark of chestnut tree where the primary infection occurred. Moreover, the *lac*3 gene was specifically down-regulated by the presence of hypovirus, CHV1.

252. Nondegradative tolerance to the phytoalexin, pisatin, in the fungal pathogen *Nectria haematococca* MPVI. Jeffrey J. Coleman and Hans D. VanEtten. University of Arizona

In plants, a major resistance mechanism is the production of antimicrobial compounds (phytoalexins) in response to the attack of pathogenic microorganisms. To avoid this host response, pathogens have evolved tolerance mechanisms that make them resistant to their host's phytoalexin. The phytopathogenic fungus, *Nectria haematococca* MPVI, has at least two known mechanisms to tolerate the phytoalexin pisatin, produced by its host, garden pea. The most extensively characterized mechanism of tolerance to pisatin in *N. haematococca* involves a one-step demethylation catalyzed by an enzyme called pisatin demethylase (pda). However, this pathogen also has a "nondegradative tolerance" (NDT) mechanism that is inducible and specific for pisatin. Preliminary results suggest that this mechanism may be the result of an efflux pump, or more specifically, an ABC transporter. ABC transporters that might be responsible for NDT have been identified using RT-PCR and degenerate primers biased to other fungal transporters on mRNA from pisatin treated mycelia of *N. haematococca*. Three putative ABC transporters have high amino acid similarity to Gpabc1 from *Gibberella pulicaris*, ABC1 from *Magnaporthe grisea*, or BcatrB from *Botrytis cinerea*. These three ABC transporters are involved in antimicrobial tolerance and/or pathogenesis. Two of these transporters were significantly up-regulated in pisatin treated mycelia as measured by real time PCR, and complementation in *Neurospora crassa* suggests at these ABC-transporters may be involved in NDT.

253. Functional analysis of the *Magnaporthe grisea ACE1* locus involved in avirulence toward *Pi33* resistant rice cultivars. Jérôme Collemare[1], Heidi U. Böhnert[1], Mikaël Pianfetti[1], Isabelle Fudal[1], Didier Tharreau[2] and Marc-Henri Lebrun[1]. [1]FRE 2579 CNRS-Bayer Cropscience, Physiologie des plantes et des champignons lors de l'infection, Bayer Cropscience, 14-20 rue P Baizet, 69263 Lyon Cedex 09, France. [2]UMR BGPI, CIRAD-INRA-ENSAM, TA41/K, 34398 Montpellier Cedex 05, France

Interactions between resistant rice cultivars and the rice blast fungus, *Magnaporthe grisea*, are often governed by gene-for-gene relationships. The avirulent isolate Guy11 carries the avirulence gene *ACE1* controlling the production of a signal specifically recognized by rice cultivars carrying the resistance gene *Pi33*. *ACE1* encodes a polyketide synthase fused to a non-ribosomal peptide, an enzyme involved in the biosynthesis of a microbial secondary metabolite. *ACE1* is specifically expressed in mature appressoria during the penetration of the fungus into host plant leaves, and the resulting enzyme is retained in the cytoplasm of appressoria. Deletion analysis of *ACE1* promoter led to the identification of a 102 bp region required for its transcriptional regulation. This region contains a putative binding site of fungal binuclear zinc finger transcription factors. Site-directed mutagenesis of this putative binding site will be used to assess its role in the regulation of *ACE1* expression. *Ace1-ks0*, a non-functional *ACE1* allele obtained by site-directed mutagenesis of an essential amino acid of the polyketide synthase KS domain, is unable to confer avirulence. This result suggests that the avirulence signal recognized by *Pi33* resistant rice is not the Ace1 protein, but is likely to be the secondary metabolite synthesized by Ace1. In order to characterize this metabolite, we are performing a metabolic profiling of *M. grisea* appressoria by LC-MS-MS, using onion epidermis infected with virulent or avirulent strains. The *ACE1* locus is 70kb long and carries 15 genes predicted to encode enzymes involved in secondary metabolism, including two enoyl-reductases, one MFS-transporter and a binuclear zinc-finger transcription factor. All these genes have the same penetration-specific expression pattern as *ACE1*, thus defining a cluster. The inactivation of these genes in an avirulent isolate is underway to evaluate their role in the biosynthesis of the avirulence signal recognized by *Pi33* resistant rice cultivars.

254. The transcription-associated proteins of *Fusarium graminearum* identified by sequence clustering and profile analyses. Richard Coulson[1], Martin Urban[2], John Antoniw[2], and Kim Hammond-Kosack[2]. [1]European Bioinformatics Institute, Wellcome Trust Genome Campus, Cambridge, CB10 1SD, UK and [2]Rothamsted Research, Herts, AL5 2JQ, UK

The trichothecene mycotoxin producing Ascomycete fungus *Fusarium graminearum* causes ear blight disease of small grain cereals. Infections lower grain quality and safety, and are of increasing global concern. In 2003, its genome was sequenced to ~10 × coverage by the Broad Institute (http://www.broad.mit.edu/annotation/fungi/fusarium). As part of the global initiative to complete the manual annotation of the genome, we have explored in depth *F. graminearum* sequences involved with the transcriptional process. Eukaryotic transcription is a highly regulated process involving interactions between large numbers of proteins, exhibiting a high degree of taxon-specificity. To identify transcription-associated proteins (TAPs), the genome was queried with a reference set of TAPs, extracted from the protein sequence databases via keyword searches (Coulson & Ouzounis (2003) Nucleic Acids Research 31, 653-660). The TRIBE-MCL algorithm was employed to detect TAP families in *F. graminearum*, in addition to those present in six model organism species: *Schizosaccharomyces pombe, Saccharomyces cerevisiae, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster*, and *Mus musculus.* Four plant pathogens *Ashbya gossypii, Magnaporthe grisea, Aspergillus oryzae, Ustilago maydis,* two human pathogens, *Candida albicans* and *Aspergillus fumigatus* and two free living saprophytes, *Aspergillus nidulans* and *Neurospora crassa* were also included in the study. The findings from this TAP-TRIBE analysis, and a second complementary approach utilising profile-hidden Markov models of domains present in well-characterised transcriptional regulators, will be presented. Currently, we are exploring the physical distribution of each TAP gene amongst the four *F. graminearum* chromosomes.

255. Analysis of Expressed Sequence Tags from *Alternaria brassicicola* infected *Brassica oleracea* var. *capitata*. R.A. Cramer Jr[1], Y. Cho [2], T.K.Mitchell[3], K.D. Craven[3], M. Thon[4], Christopher B. Lawrence[2]. [1] Current Address Duke University Medical Center, Durham, NC. [2] Virginia Bioinformatics Institute, Blacksburg, VA. [3] North Carolina State University, Raleigh, NC. [4] Texas A&M University, College Station, TX.

Alternaria brassicicola (AB) is a necrotrophic fungus that causes black spot disease on economically important Brassicas such as cabbage and is a model pathogen for studies with Arabidopsis. The purpose of this study was to identify genes involved in the interaction between AB and *Brassica oleracea* var. *capitata* (BO). A functional genomics approach was used to identify candidate pathogenicity genes by creating a suppression subtractive hybridization (SSH) library enriched for AB and BO genes expressed during pathogenesis. A total of 4224 expressed sequence tags (ESTs) were sequenced and assembled into a 3112 unisequence set using the assembly program CAP3. This unisequence set contained 608 contigs and 2504 singletons. The library had an estimated redundancy rate of 26%. BLAST algorithms were used to search publicly available databases to gain putative identities of the ESTs. BLAST searches identified 312 of the sequences as AB, 1614 as BO, and 1186 as unknown. A similar analysis was conducted on a cDNA library created from AB nitrogen starved (NS) mycelia with an approximate 40% redundancy rate. This full length cDNA library contained 1660 unisequences (253 contigs and 1407 singletons). NS sequences proved useful in identifying unknown sequences in the BO library. Many ESTs from the infected BO and NS libraries had homology with known fungal pathogenicity factors. RT-PCR was used to confirm the differential expression of 10 putative fungal pathogenicity factors and 10 host genes in planta. Results of these experiments will be presented.

256. Cloning and Characterization of a Cyanide Hydratase from the Necrotropic Fungal Pathogen *Alternaria brassicicola*. R.A. Cramer Jr[1], Juan Wang, Josh Davis[2], Christopher B. Lawrence[2]. [1] Current Address Duke University Medical Center, Durham, NC. [2] Current Address Virginia Bioinformatics Institute, Blacksburg, VA.

In order to colonize and infect their hosts, plant pathogenic fungi must overcome potentially toxic compounds produced by plants. Previously a cDNA clone with homology to a cyanide hydratase enzyme was identified in a suppression subtracted library of *Alternaria brassicicola* infected *Arabidopsis*. In this study, random amplification of cDNA ends was used to clone the full length cyanide hydratase cDNA sequence which was named ACH (Alternaria Cyanide Hydratase), This sequence was used to disrupt the genomic cyanide hydratase locus using a single homologous recombination event. Mutants with a disrupted ACH locus displayed normal vegetative growth in vitro, normal conidiation, and increased sensitivity in vitro to KCN. Lesion diameters, in developed lesions, on infected *Brassica oleraceae* var. *capitata* leaves were similar for wild type *A. brassicicola* and ach mutants. However, there was a significant difference in the number of lesions that developed between wild type and ach mutants suggesting a possible role in fungal pathogenicity for ACH. Bioinformatic analysis of available fungal genomes revealed that cyanide hydratase homologs are found in fungi with diverse life styles including saprophytes and pathogens. Thus, the exact role in fungal biology that cyanide hydratase enzymes play is still not clear.

257. **Signal Transduction and Hydrophobin Gene Expression in the Maize Pathogen *Cochliobolus heterostrophus*.** Ofir Degani[1], Sophie Lev[1], Mark S. Rose[2] and Benjamin A. Horwitz[1]. [1]Department of Biology, Technion–Israel Institute of Technology, Haifa 32000, Israel; [2]Syngenta Biotechnology Inc., Research Triangle Park, NC 27709.

Filamentous fungi produce hydrophobins, small proteins that are localized on the outer surface of their cell walls and are involved in growth and development. Nutrient availability and light regulate hydrophobin gene expression. Recently it was demonstrated that signal transduction components such as a MAPK in *Magnaporthe grisea* and a G-protein alpha subunit in *Cryphonectria parasitica* are also involved. Here we conducted a comparative study on the effect of loss of function mutations in different signaling components on hydrophobin gene expression in *Cochliobolus heterostrophus*. Furthermore, a mutant deficient in both G-protein alpha and beta subunits (Cgab1) was constructed. This mutant has an albino phenotype, lacked conidia but still formed abortive pseudothecia in a backcross, and was unable to infect maize. Loss of the G-alpha subunit led, in some conditions, to increased hydrophobin expression, which may be involved in the spore aggregation phenotype of this mutant. Mutations in the G-beta subunit led to increased hydrophobin class II expression on liquid media, while MAPK mutants or cgab1 double mutants showed reduced expression of hydrophobins and was found to have a wettable colony phenotype in their early growth stage. Together, these findings suggest an important role for MAPK and for G-protein alpha and beta subunits in mediating hydrophobin gene expression.

258. Identification of Arabidopsis genes that support parasitic symbiosis by *Peronospora parasitica*. Terrence P. Delaney[1] and Nicole M. Donofrio.[2] [1]The University of Vermont, Department of Botany, Burlington VT, USA. [2]North Carolina State University, Fungal Genomics Laboratory, Raleigh NC, USA.

Pathogens that establish intimate, non-lethal relationships with their hosts may employ strategies for host immune system evasion, and may manipulate host metabolism or physiology to benefit the parasite without destroying host tissue. We are examining infection of *Arabidopsis thaliana* by the oomycete *Peronospora parasitica* to search for these parasite activities, which may constitute essential yet poorly recognized virulence functions. Using gene expression profiling methods, we identified plant genes whose expression is altered specifically by virulent strains of *P. parasitica*, but are not linked to defense responses. Such genes are candidates to be those manipulated by the pathogen for its own benefit. We obtained T-DNA insertion mutations in some of these genes, and are now performing functional tests to determine whether the genes play a role in supporting growth of this compatible biotrophic pathogen. In a companion study, we performed mutant screens in Arabidopsis *nim1-1* mutants, which are compromised in expression of systemic acquired resistance and thus highly susceptible to a virulent strain of *P. parasitica*. Several mutants were recovered that supported less growth of the pathogen without the induction of known defense-related genes, suggesting that these plants may lack gene products important for pathogen vitality.

259. Fpr1, a member of the pathogenesis-related (PR) protein superfamily is required for virulence of *Fusarium oxysporum* on mammals. Rafael C. Prados-Rosales[1], Raquel Roldán-Rodríguez[1], Montserrat Ortoneda[2], Josep Guarro[2], and Antonio Di Pietro[1] [1]Departamento de Genetica, Universidad de Cordoba, Campus de Rabanales Ed. C5, 14071 Cordoba, Spain. [2]Unitat de Microbiologia, Facultat de Medicina i Ciencies de la Salut, Universitat Rovira i Virgili, 43201 Reus, Tarragona, Spain

The pathogenesis-related (PR) protein superfamily is widely distributed in eukaryotic organisms. While their precise biological activity remains elusive, PR proteins have been implicated in a variety of processes, including the plant defense and the mammalian immune response. We have examined the role of Fpr1, a PR-like protein of the soilborne fungus *Fusarium oxysporum*, the causal agent of vascular wilt disease in plants and an emerging opportunistic pathogen of humans. The *fpr1* gene was expressed at high levels in actively growing hyphae of *F. oxysporum*. The presence of a predicted N-terminal signal peptide suggests that FPR1 is co-translationally translocated to the ER, although GFP-tagged Fpr1 protein was found to accumulate intracellularly in spherical organelles. Targeted knockout mutants showed no detectable changes in vegetative growth or virulence on tomato plants. In contrast, the *fpr1* knockout mutants exhibited markedly reduced virulence in a disseminated immunodepressed mouse model. Virulence was fully restored by re-introduction of a wild type *fpr1* allele, but not of a $fpr1^{mut}$ allele in which two conserved residues of the predicted active site of the PR-1 protein family, His170 and Gln177, were changed to Leu and Ala, respectively. Our current efforts are directed towards the elucidation of the biochemical function of Fpr1.

260. Genomic architecture of *ftf1* and *ftf2* genes in *F. oxysporum*. B. Ramos, M.A. García-Sánchez, N. Martín-Rodrigues, A.P. Eslava and J.M. Díaz-Mínguez. Área de Genética. Centro Hispano-Luso de investigaciones Agrarias (CIALE). Universidad de Salamanca. 37007, Salamanca, Spain.

We have previously reported the isolation of *ftf1* (Fopta1a) and *ftf2* (Fopta1b), two genes coding for highly homologous transcription factors differentially involved in pathogenicity in *F. oxysporum* f. sp. *phaseoli* (Kendrick & Snyder). Both transcription factors contain a Zn(II)-Cys6 binuclear cluster DNA-binding motif and a fungal transcription factor domain. The structural region of both genes share a 80-85% of homology at the nucleotide level; this homology falls to a 70% in the promoter region. The *ftf1* gene is present only in highly virulent strains and show a dramatic peak of expression 24 to 48 h after infection of common bean plants (*Phaseolus vulgaris* L.). On the contrary, *ftf2* is present in all tested strains (pathogenic and non-pathogenic) and shows a very low level of expression in vivo and in vitro.
We have detected up to four copies of *ftf1* in highly virulent strains, while *ftf2* is a single copy gene in all the strains tested. Genomic architecture is also different in both genes. Some of the copies of *ftf1* show some restriction fragment length polymorphisms, but all of them are closely linked to the only copies of transposon marsu detected in *F. oxysporum* f.sp. *phaseoli*. The single copy of *ftf2* is located downstream of an homologue of *bimB3*, a gene involved in coupling DNA replication with mitosis in *Aspergillus nidulans*.

261. A *Botrytis cinerea* gene induced *in planta* encodes a protein with homology with cyclins. Benito Pescador, D., Martín-Domínguez, R., Díaz-Mínguez, J.M., Eslava A. P. and Benito E.P. Area de Genética. Centro Hispano-Luso de Investigaciones Agrarias. Universidad de Salamanca. Edificio Departamental. Campus Unamuno, s/n. 37007 Salamanca. SPAIN.

The causal agent of grey mold, *Botrytis cinerea* Pers. (teleomorph: *Botrytinia fuckeliana* (de Bary) Whetzel) is a filamentous fungus with a broad host range and responsible of important economic losses. A large number of research groups have been working intensively on the characterization of the fungal pathogenicity mechanisms and on the design of control strategies, either chemical or biological.
In order to understand the molecular mechanisms involved in the infection process of *B. cinerea*, an experimental approach based on the analysis of differential gene expression during the plant/fungus interaction was applied. This analysis allowed us to detect different cDNA fragments derived from *B. cinerea* genes whose expression is induced *in planta*. The gene encoding one of these fragments, named *Bde2*, is being characterized in detail.
Using as a probe the cDNA fragment initially detected, it was shown by Northern blot hybridization analysis that gene *Bde2* is expressed only during late stages of the infection process, specifically during colonization and maceration of the infected tissue. Southern analysis demonstrated it is a single copy gene. The full-length genomic copy of *Bde2* was cloned from a genomic library and its structure and sequence was determined. Sequence analysis and public databases searches revealed an ORF translation of which generates a protein with significant homology to cyclins.
To get deeper insights into the role of the *Bde2* gene, and of the encoded protein, in the infection process of *B. cinerea*, a functional characterization approach based on the isolation and characterization of mutants specifically altered in gene *Bde2* was designed. Transformation experiments are being carried out in order to obtain *Bde2* gene replacement mutants using a plasmid carrying a mutant allele in which the coding region of the wild type allele has been replaced by a gene fusion expressing the bacterial gene conferring hygromicin resistance under the control of fungal promoter and terminator sequences. Transformants lacking gene *Bde2* will be analyzed for alterations during growth *in planta* and during saprophytic growth.

262. The melanin biosynthetic gene *PKS1* is dispensable for virulence of the banana pathogen *Mycosphaerella fijiensis*. Bruno Giuliano Garisto Donzelli and Alice C.L. Churchill, Department of Plant Pathology, Cornell University, Ithaca, NY, USA.

Black leaf streak disease (BLSD) or black Sigatoka, caused by *Mycosphaerella fijiensis*, is the most destructive disease of bananas and plantains (*Musa* sp.) worldwide. Shunt metabolites from the DHN-melanin pathway, such as juglone and 2,4,8-trihydroxytetralone, have been hypothesized to play a role in the disease as phytotoxins causing leaf chlorosis and necrosis. We cloned a polyketide synthase gene (*PKS1*) responsible for the first step of the DHN-melanin pathway in *M. fijiensis* and generated melanin-deficient mutants of the fungus by both targeted gene knockout (KO) and RNA-mediated silencing. Targeted gene KO was accomplished using a construct in which the Hygromycin resistance cassette was flanked by ca. 1 and 3 Kb of the *PKS1* gene. RNA-mediated gene silencing was achieved by expressing an inverted repeat fragment of the *PKS1* gene. Both constructs were delivered to a wild type virulent strain by *Agrobacterium tumefaciens*-mediated transformation. Both the *PKS1*-KO isolates and the *PKS1*-silenced isolates displayed similar reduced pigmentation phenotypes. Pathogenicity assays on the susceptible banana cultivar Grand Naine indicated no detectable alteration in virulence of two KO mutants. In contrast, several RNA-silenced mutants showed reductions in both melanization and virulence. Chemical analyses of the virulent *PKS1*-KO isolates indicated that production of melanin-DHN pathway shunt metabolites was abolished in the mutants, suggesting that these metabolites are dispensable for *M. fijiensis* virulence. This is a first report of gene disruption and silencing in a *Mycosphaerella* pathogen of bananas and the first genetic evidence to suggest that melanin shunt metabolites do not play roles in BLSD.

263. Towards the development of a large–scale transposon insertional mutagenesis across the *Fusarium graminearum* genome. Marie Dufresne, Sarrah Ben M'barek, Gert H.J. Kema and Marie-Josée Daboussi. Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France

The *mimp1* element is a miniature inverted-repeat transposable element (MITE) previously identified in *Fusarium oxysporum* and shown to be mobilized by the transposase of a *Tc1-mariner member*, impala. Analysis of *mimp1* distribution in *Fusarium* strains revealed that it is present in all *F. oxysporum* strains and in most of related species such as *F. redolens* and *F. hostae*. On the contrary, this element is absent from more distant species such as *F. culmorum* and *F. graminearum*.
Here, we show that the double component system *mimp1/impala* transposase is fully functional in *F. graminearum*. Transposition characteristics (TA target site, duplicated upon *mimp1* insertion, excision footprints) were found to be the same as in the original host species. Moreover, as already observed in *F. oxysporum*, *mimp1* reinserted very frequently (around 95%) allowing the generation of an efficient collection of revertants.
Taking advantage of the availability of the F. graminearum genome sequence and using TAIL-PCR, we started to recover sequences flanking *mimp1* reinsertions in a collection of revertants obtained from several initial transformants. Preliminary results obtained from a set of 25 revertants indicated that *mimp1* tends to reinsert in 5'-non coding regions, 40% of the insertion events taking place less than 1000 bp upstream the initiation codon of a predicted ORF.
Such results are very promising for the development of this novel double component system as a powerful mutagenesis tool on a high throughput scale in *F. graminearum* as well as other ascomycete fungi.

264. Determining the role of reactive oxygen species generation in *Magnaporthe grisea*. Martin Egan and N Talbot. University of Exeter, Devon, UK

NADPH oxidases (Nox) are enzymes used to generate reactive oxygen species (ROS). Here we investigate the role of NADPH oxidase-generated ROS in the infection related development of the phytopathogenic fungus *Magnaporthe grisea*. *M.grisea* parasitizes more than fifty species of grasses, but is best known as the causal agent of rice blast disease. The formation of a specialised cell known as the appressorium allows the fungus to breach the host cuticle and subsequently cause infection. Using the super oxide detector dihydrofluorescein diacetate, we have monitored the production of ROS during conidial germination and report the occurence of an oxidative burst during appressorium formation. We also report the accumulation of ROS in hyphal tips. We demonstrate that appressorial development is inhibited by DPI, an NADPH oxidase inhibitor.
We have also characterised *NOX1*, one of two genes encoding NADPH oxidases in the *M. grisea* genome. Through targeted gene replacement, we have shown that *NOX1* cannot cause disease and is required for pathogenicity. Delta*nox1* mutants also exhibit aberrant germ tube morphology and frequently produce multiple appressoria. Strikingly normal hyphal growth appears unaffected in delta*nox1* mutants indicating that ROS generation is associated with cellular differentiation.

265. Characterization of the *MST7* MAP kinase kinase gene in *Magnaporthe grisea*. Xinhua Zhao, Yangseon Kim and Jin-Rong Xu. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

Many fungal pathogens invade plants using specialized infection structures called appressoria. In the rice blast fungus *Magnaporthe grisea*, the *PMK1* MAP kinase is essential for appressorium formation and invasive growth. We functionally characterized the *MST7* and *MST11* genes of *M. grisea* that are homologous to the yeast MAP kinases kinase *STE7* and MAP kinases kinase kinase *STE11*. Similar to the *pmk1* mutant, the *mst7* and *mst11* deletion mutants were non-pathogenic and failed to form appressoria. When a dominant allele $MST7^{S212D\ T216E}$ was introduced into a *mst11* mutant, the resulting transformants formed appressoria but failed to penetrate and infect rice leaves, indicating that constitutively active *MST7* only partially rescued the defects of the *mst11* mutant. Although the interaction between Mst7 and Mst11 is weak in yeast two-hybrid assays, both of them directly interact with Mst50, a putative adapter protein for the Pmk1 MAPK cascade. Interesting, a putative MAPK docking site is well-conserved in *MST7* and its homologs. The $MST7^{12\text{-}20}$ allele failed to complement the *mst7* mutant, suggesting that this docking site may be essential for the interaction between Mst7 and Pmk1. The role of this MAPK docking site and the interaction of Mst7 with Pmk1, Mst11, and Mst50 will be further characterized.

266. A Precocious Sporulation mutant of *Leptosphaeria maculans* has altered pathogenicity on *Brassica napus*. Candace Elliott and Barbara J. Howlett, The School of Botany, University of Melbourne, VIC Australia

Blackleg disease, caused by the ascomycete, *Leptosphaeria maculans*, is the most serious disease of canola (*Brassica napus*) worldwide. Knowledge of both plant defence mechanisms and fungal disease processes (pathogenicity) will allow development of novel control strategies. I have used an Agrobacterium-mediated random mutagenesis approach to generate isolates with a reduced ability to infect *B. napus*. Of 353 mutants screened on *B. napus* cotyledons and stems, 4 showed reduced lesion size. Sequencing of flanking regions revealed one isolate with an insertion in a hypothetical protein of unknown function. Three other isolates contained single T-DNA insertions in genes encoding a zinc-finger protein, a histone H4 gene and an alcohol dehydrogenase (ADH)-like gene. A detailed analysis of the ADH-like mutant revealed the T-DNA insertion did not disrupt expression of the ADH-like gene, but caused constitutive expression of the ADH-like gene and the adjacent thiolase gene. This has resulted in a mutant that attains competence for sporulation at an earlier stage than wild type but is unable to produce lesions with the same frequency as wild type on canola cotyledons and stems. Experiments including complementation, over-expression and gene knockout are currently underway to elucidate whether the ADH-like or the thiolase gene is responsible for this alteration in sporulation and pathogenicity.

267. The location of *Sphaeropsis sapinea*, the causal agent of pine tip blight, in latently infected versus symptomatic Austrian pine shoots. Jennifer L. Flowers, John R. Hartman, and Lisa J. Vaillancourt; Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546

Sphaeropsis tip blight is a common disease that affects many conifers worldwide. Symptoms of *Sphaeropsis sapinea* infection include stunted shoots with necrotic, stunted needles, resinous cankers, and a general decline of the tree. These symptoms lead to significant economic losses of pines in managed plantings. Latent infections of *S. sapinea* are common in current-year symptomless Austrian pine trees, as well as within apparently healthy branches on diseased pines. Dissection and culturing of latently infected pine shoots suggested that *S. sapinea* was located in the region between the inner bark and vascular cambium. In contrast, the fungus could be recovered from all tissue types in shoots with symptoms of tip blight. Microscopy was conducted to further define the location of the pathogen in latently infected shoots, and to compare and contrast latent versus symptomatic infections. Diseased, latently infected, and uninfected Austrian pine shoots were differentiated using a combination of visible symptoms and a *S. sapinea*-specific nested-polymerase chain reaction protocol. The shoot samples were embedded in Spur's resin, and 1 micron thick sections were cut, stained with toluidine blue, and observed under the microscope. Large numbers of fungal hyphae were observed throughout the tissues of diseased pine shoots, while no hyphae were observed in uninfected pine shoots. In some of the latently infected shoots, fungal hyphae were observed in small pockets of collapsed periderm cells. A common assumption in tree pathology is that latent infections can transform into pathogenic ones when the host is physiologically stressed. We are continuing our observations as part of our effort to understand the nature of this transformation from latent to pathogenic infection, with the hope that this may lead to more effective management of this devastating disease.

268. Impaired purine biosynthesis affects pathogenicity of *Fusarium oxysporum* f. sp. *melonis*. Youlia Denisov[1,2], Oded Yarden[1] and Stanley Freeman[2]. [1]Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100; [2]Department of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel

The vascular wilt pathogen *Fusarium oxysporum* f.sp. *melonis* causes worldwide yield losses of muskmelon. In this study we characterized a UV-induced non-pathogenic mutant (strain 4/4) of *F. oxysporum* f.sp. *melonis*, previously identified as a potential biological control agent. During comparative analysis of vegetative growth parameters using different carbon sources, strain 4/4 showed a delay in development and secretion of extracellular enzymes, compared to the wild type strain. Amendments of the growth medium with yeast extract, adenine or hypoxanthine, but not guanine, complemented the growth defect of strain 4/4, as well as secretion and partial activity of cellulases and endopolygalacturonases, indicating that the strain is an adenine auxotroph. Incubation of strain 4/4 conidia in adenine solution, prior to inoculation of muskmelon plants, partially restored pathogenicity to the mutant strain. As part of the characterization of pathogenicity factors of Fusarium wilt, a collection of approx. 2000 Agrobacterium-transformed mutants were generated and screened for pathogenicity on melon plants. At this stage, five putative impaired pathogenicity mutants are being characterized.

269. A defect in a NirA-like transcription factor confers morphological abnormalities and lack of pathogenicity in *Colletotrichum acutatum*. Sigal Horowitz [1,2], Stanley Freeman [2], Aida Zveibil [2], and Oded Yarden [1]. [1] Department of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100; [2] Department of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel.

A nonpathogenic REMI mutant of *Colletotrichum acutatum*, designated Ca-5, was isolated whereby in the absence of an external nitrogen source exhibited extended germ tube growth prior to appressoria formation on solid surface and strawberry leaf. Ca-5 exhibited restricted hyphal growth and did not cause lesions on plants but grew necrotrophically when inoculated directly onto wounded sites. The deduced amino acid sequence of the REMI-impaired gene product, designated Nir1, is highly similar to the *Aspergillus nidulans* NirA protein, a transcriptional regulator of nitrogen metabolism. Inoculation of leaves with wild type or Ca-5 conidia in the presence of a nitrogen source resulted in massive epiphytic hyphal production, appressoria formation and rapid symptom development. The nutritional status of *C. acutatum* at an early stage of colonization and appressoria formation was assessed by following the expression of nitrate reductase (NR) and glutamine synthetase (GS) in different media. Under all growth conditions there was no effect on GS, however, NR was induced by nitrate and repressed by a rich medium. In addition, NR transcription increased at the appressoria stage, indicating that nitrogen starvation constitutes a cue for regulation of appresoria development. Our results suggest that nitrogen starvation stimulates synchronous preinfection development which is lacking in Ca-5.

270. Characterisation of a P-type ATPase in *Magnaporthe grisea* defines an exocytotic pathway required for fungal pathogenicity. Martin J.Gilbert, Christopher R.Thornton, Nicholas J.Talbot. School of Biological Sciences, University of Exeter, Washington Singer Laboratories, Perry Road, Exeter, EX4 4QG, United Kingdom.

The ability to secrete protein products into host cells is a fundamental characteristic of pathogenic organisms. In bacterial pathogens, the type III secretion system is utilised to deliver these effector proteins into host cells during infection. It is not known, however, how fungal pathogens achieve delivery of effector proteins during disease establishment or whether they possess specialised secretion systems that have evolved for this purpose.Here we report that a mutant of the rice blast fungus *Magnaporthe grisea*, *deltaMgapt2*, that is affected in exocytosis, is also compromised in its ability to cause disease. *MgAPT2* encodes an aminophospholipid translocase (APT), one of four putative APTs present in the *M. grisea* genome. This family of APTs includes the previously characterised *PDE1* gene, a virulence factor for rice blast disease (Balhaldere & Talbot 2000. Plant Cell, 13, 1987-2004). *deltaMgapt2* mutants accumulate abnormal Golgi cisternae. These appear as double-membrane ring structures similar to Berkeley bodies. *deltaMgapt2* mutants grow, sporulate and form appressoria normally but are unable to grow on several carbon sources including starch and glycogen. We have shown that the ability to secrete a subset of extracellular enzymes is impaired in *deltaMgapt2* mutants. The product of *MgAPT2* is found in the Golgi network indicating a role in vesicle docking during exocytosis. We discuss the implications of this finding for our knowledge of fungal pathogenesis.

271. Identification of Novel Pathogenicity Genes by Macroarray Analysis of the Botrytis-arabidopsis Interaction. Anastasia Gioti, Adeline Simon, Muriel Viaud, Jean-Marc Pradier,Caroline Levis. Unité de Phytopathologie et Méthodologies de la Détéction (P.M.D.V), I.N.R.A de Versailles, Route de St-Cyr, 78026, Versailles Cedex, France.

Our group is investigating the molecular mechanisms of pathogenicity of *Botrytis cinerea*, an ascomycete with a broad spectrum of plant-hosts. Here we present results of the expression profiling of 3.032 fungal genes, spotted onto macroarray filters, during the interaction of *Botrytis* with *Arabidopsis thaliana*.
In order to follow the kinetics of fungal gene expression *in planta* , we hybridized the macroarrays with RNAs extracted from the infected plant leaves at three different stages of infection. As "reference" probes we used RNAs of *Botrytis* grown *in vitro*, as well as the RNAs of the uninfected plant. The resulting data were treated statistically (GeneAnova, P.C.A) and clustered (Genesis). 29 genes were shown to be systematically induced at specific infection stages and over-expressed during infection compared to the *in vitro* growth reference.
From the above, eight genes were chosen for a reverse genetic analysis aiming to confirm their role in the pathogenicity of *Botrytis*. Knock-out mutants were obtained for two of these genes so far. Their phenotype during pathogenicity tests on different plants will be presented. In overall, our results show that the transcriptomic study of the fungus *in planta* is possible and permits us to identify new genes involved in the infection process.

272. Cell surface mannan and the Candida-host interaction. Gow, N.A.R., Bain, J.M, Bates, S., Bertram, G., Hobson, R.P., Hughes, H.B., Munro, C.A., MacCallum, D., Odds, F.C., Brown, A.J.P. Aberdeen Fungal Group, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, UK.

The outer layer of the cell wall of *Candida albicans* is heavily enriched in glycosylated proteins that play critical roles in cell adherence, and act as major antigens and in the immunoregulation of the host. A null mutant in the Golgi manganese transporter gene PMR1 was viable in vivo, had greatly reduced N- and phosphomannan and was attenuated in virulence. Therefore normal mannosylation is required for pathogenesis. The *C. albicans* O-linked mannan consists of a pentasaccharide in which Mnt1p and Mnt2p participate as partially functionally redundant enzymes in the assembly of the terminal two a-1,2-mannose residues. Deletion of either MNT1, MNT2 or both MNT1 and MNT2 resulted in strains with reduced adherence to epithelia and attenuation of virulence. This suggests that O-mannan functions as a ligand in interactions with host surfaces. Mutants with deletions in the MNN4 gene are almost devoid in phosphomannan, which has been implicated in recognition of *C. albicans* by macrophages, but were phagocytosed normally. Deletion of the OCH1 resulted in elimination of the outer N-mannan chains, induction of the cell wall salvage pathway and loss of virulence. Analysis of glycosylation mutants deomonstrates that the carbohydrate epitopes of mannoproteins play key roles in pathogenesis of *C. albicans*.

273. Siderophore biosynthesis but not reductive iron assimilation is essential for *Aspergillus fumigatus* virulence. Markus Schrettl[1], Elaine Bignell[2], Claudia Kragl[1], Chistoph Jöchl[1], Tom Rogers[2], Herbert N. Arst Jr[2], Ken Haynes[2] and Hubertus Haas[1]. [1]Department of Molecular Biology, Medical University Innsbruck, Austria; [2]Department of Infectious Diseases, Imperial College London, UK.

The ability to acquire iron in vivo is essential for most microbial pathogens. Here we show that *Aspergillus fumigatus* does not have specific mechanisms for the utilization of host iron sources. However, it does have functional siderophore-assisted iron mobilization and reductive iron assimilation systems, both of which are induced upon iron deprivation. Abrogation of reductive iron assimilation, by inactivation of the high-affinity iron permease (FtrA), has no effect on virulence in a murine model of invasive aspergillosis. In striking contrast, *A. fumigatus* L-ornithine-N5-monooxygenase (SidA), which catalyses the first committed step of hydroxamate-type siderophore biosynthesis, is absolutely essential for virulence. Thus, *A. fumigatus* SidA is an essential virulence attribute. Combined with the absence of a sidA ortholog - and the fungal siderophore system in general - in mammals, these data demonstrate that the siderophore biosynthetic pathway represents a promising new target for the development of antifungal therapies.
This work was supported by the Austrian Science Foundation), the Chronic Granulomatous Disorder Research Trust and the Biotechnological and Biological Sciences Research Council.

274. *GzGRR1* encoding a putative F-box protein is involved in pathogenesis and sexual development by *Gibberella zeae*. You-Kyoung Han[1], Sung-Hwan Yun[2], and Yin-Won Lee[1]. [1]School of Agricultural Biotechnology, Seoul National University, Seoul 151-921; [2]Division of Life Sciences, Soonchunhyang University, Asan, 336-745, Korea

Gibberella zeae is an important pathogen of cereal crops in many areas of the world, causing head blight of small grains including corn, wheat, barley, and rice. In addition, this fungus produces mycotoxins such as trichothecenes and zearalenone on diseased crops and has been a potential threat to human and animal health. To identify pathogenesis-related genes, we selected several *G. zeae* mutants defective for the traits involved in disease development. The mutant ZH436, generated by restriction enzyme-mediated integration, showed significantly reduced virulence toward host plants along with other pleiotropic phenotypes such as reduced hyphal growth on nutrient rich conditions and no sexual development. In addition, this mutant produced incomplete tetrads with aberrant morphology when outcrossed to a *mat1-2* deletion strain. Molecular characterization revealed that vector insertion point was located within the ORF, designated *GzGRR1* showing a high similarity to *GRR1*, a regulator for glucose repression in *Sacharomyces cerevisiae*; the translation product of *GzGRR1* carries both a putative F-box and a leucine-rich repeats (LRR) domain. Northern blot analysis showed that *GzGRR1* was constitutively expressed but the transcript was highly produced during the perithecial stage. These results suggest that *GzGRR1*, as other F-box proteins, may be involved in degradation of proteins by ubiquitination, specifically those required for virulence or sexual development in *G. zeae*.

275. Oomycete zoospores secrete adhesins containing thrombospondin type-1 repeats. Andrea V. Robold and Adrienne R. Hardham. Plant Cell Biology Group, Research School of Biological Sciences, The Australian National University, Canberra, ACT 2601, Australia.

Adhesion is a key aspect of the establishment of disease by pathogens of animals and plants. Adhesion anchors the parasite to the host surface and is often a prerequisite for the differentiation of specialized infection structures and host invasion. A number of adhesin molecules produced by microbial pathogens infecting animals have been characterised, however, to date molecular details of adhesins of plant pathogens, especially fungi, are largely restricted to general descriptions of the nature of heterogeneous secreted materials. Many devastating plant diseases are caused by oomycetes, fungus-like organisms that include *Phytophthora infestans*, responsible for late blight of potato. In this presentation we describe the cloning and characterisation of a gene encoding a *Phytophthora* adhesin and the discovery that the adhesin protein contains 47 copies of the thrombospondin type-1 repeat, a motif found in adhesin proteins in animal cells and apicomplexan (e.g. malarial) parasites but not in plants, green algae or true fungi. Our results give the first detailed information on an adhesin of a fungal or fungus-like plant pathogen, and highlight intriguing similarities in structural and molecular features of host attachment in oomycete and apicomplexan parasites.

276. Identification of AM-toxin biosynthesis gene cluster in the apple pathotype of *Alternaria alternata*. Yoshiaki Harimoto[1], Rieko Hatta[1], Motoichiro Kodama[2], Mikihiro Yamamoto[3], Hiroshi Otani[3] and Takashi Tsuge[1]. [1]Graduate School of Bioagricultural Science, Nagoya University, [2]Faculty of Agriculture, Tottori University, [3] Faculty of Agriculture, Okayama University, Japan.

The apple pathotype of *Alternaria alternata* produces host-specific AM-toxin and causes Alternaria blotch of apple. We previously isolated two genes, *AMT* and *AMT2*, involved in AM-toxin biosynthesis and found that these genes are encoded by conditionally dispensable (CD) chromosomes of the apple pathotype strains. In this study, we conducted the expressed sequence tag (EST) analysis of the 1.4-Mb chromosome encoding *AMT* genes in strain IFO08984. A cDNA library (41,000 clones) of IFO08984 was screened with the 1.4-Mb chromosome probe, and 270 clones were isolated. Sequence analysis showed that 270 clones are derived from 148 unique genes. Database homology search detected 22 candidate genes, which are possibly involved in AM-toxin biosynthesis. To identify AM-toxin biosynthesis gene cluster, BAC clones encoding the *AMT* genes and the candidate genes were isolated. Structural analysis of a 118-kb insert of a BAC clone (AM-BAC1) detected *AMT*, *AMT2*, 16 candidate genes, and additional 19 putative genes. Expression analysis of these genes by real-time RT-PCR showed that *AMT*, *AMT2*, and eight genes were specifically up-regulated in AM-toxin production medium. All the up-regulated genes are present within the 62-kb region in AM-BAC1, suggesting that this region is responsible for AM-toxin biosynthesis.

277. Partial structure of the conditionally dispensable (CD) chromosome controlling AF-toxin biosynthesis and pathogenicity in the strawberry pathotype of *Alternaria alternata*. Rieko Hatta[1], Akihisa Shinjo[1], Yoshiaki Harimoto[1], Mikihiro Yamamoto[2], Kazuya Akimitsu[3], and Takashi Tsuge[1]. [1]Graduate School of Bioagricultural Sciences, Nagoya University, [2]Faculty of Agriculture, Okayama University, [3]Faculty of Agriculture, Kagawa University, Japan.

The strawberry pathotype of *Alternaria alternata* produces host-specific AF-toxin and causes black spot of strawberry. We previously isolated AF-toxin biosynthesis genes (*AFT* genes) from strain NAF8. The *AFT* genes were found to be clustered on a 1.05-Mb CD chromosome of NAF8, which is dispensable for saprophytic growth in culture. We conducted expressed sequence tag (EST) analysis of the 1.05-Mb chromosome. A cDNA library of NAF8 was screened with the 1.05-Mb chromosome probe, and 342 clones were isolated. Sequence analysis showed that 342 clones are derived from 97 unique genes, and that 11 genes correspond to known *AFT* genes. We analyzed structure of the 1.05-Mb chromosome. An 8X whole chromosome shotgun sequence assembly of the 1.05-Mb chromosome was obtained, and 150-kb and 420-kb contigs were identified. The 150-kb contig contains 19 *AFT* genes and 11 transposon-like sequences. In contrast, the 420-kb contig contains no *AFT* genes and encodes 94 putative ORFs. Database homology search of these ORFs detected no housekeeping gene homologs. These results suggest that the 420-kb region is not essential for AF-toxin biosynthesis and saprophytic growth.

278. Distinct signaling pathways regulate plant cell death induced by INF1, CRN2 and PiNPP1.1 of *Phytophthora infestans*. Edgar Huitema, Cahid Cakir, Thirumala-Devi Kanneganti, Natalia Norero and Sophien Kamoun. Department of Plant Pathology, The Ohio State University-OARDC, Wooster, OH 44691

Phytophthora infestans, a plant pathogenic oomycete, causes late blight on potato and tomato. Most plant species display active defense responses upon *P. infestans* infection and are fully resistant (nonhost resistance). The mechanism that underlies nonhost resistance is hypothesized to involve recognition of *P. infestans* effectors. Perception of these proteins initiates a series of discrete signaling steps, resulting in cell death and defense responses. Previously, *P. infestans* INF1, CRN2 and PiNPP1.1 were identified as necrosis inducing proteins. To expand our understanding of resistance, we investigated various aspects of INF1, CRN2 and PiNPP1.1-induced cell death. First, we used virus induced gene silencing (VIGS) to silence 35 signaling genes in *N. benthamiana* and measure their impact on elicitor induced necrosis. Second, we tested whether AVRPTOB, a suppressor of AVRPTO-induced cell death, suppresses the activity of INF1, CRN2 and PiNPP1.1. Third, we applied combinations of INF1, CRN2 and PiNPP1.1 in agroinfiltration assays to test whether cross-talk occurs between signaling pathways. Our results point to at least two distinct cell death pathways. This work is helping us dissect nonhost resistance to this economically important pathogen.

279. EST analysis during conidiation of the plant pathogenic fungus *Fusarium oxysporum*. Yuichiro Iida, Toshiaki Ohara, and Takashi Tsuge. Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.

Fusarium oxysporum is a soil-borne facultative parasite that causes economically important losses on a wide variety of crops. *F. oxysporum* produces three kinds of asexual spores, microconidia, macroconidia, and chlamydospores. Falcate macroconidia are generally formed from terminal phialides on conidiophores. Ellipsoidal microconidia are formed from intercalary phialides on hyphae. Globose chlamydospores with thick walls are developed by the modification of hyphal and conidial cells. Here we describe expression sequence tag (EST) analysis during conidiation of *F. oxysporum*. *F. oxysporum* produces macroconidia and microconidia in carboxymethyl cellulose liquid medium (CMC) but not in complete liquid medium (CM). The cDNA libraries were constructed using mRNAs from fungal tissues grown in CM and CMC. The 5• f ends of 1,352 and 1,289 cDNA clones from CM (vegetative growth) and CMC (conidiation) libraries, respectively, were sequenced, and cDNAs derived from 543 and 542 unique genes were identified. Number of common genes in both libraries was only 112 (20%), suggesting that the vegetative growth and conidiation libraries had different patterns of gene expression. A total of 430 genes specific for the conidiation library were subjected to macroarray analysis, and 173 genes were identified to be up-regulated during conidiation. Expression analysis of these genes by real-time RT-PCR detected 54 genes that are markedly up-regulated during conidiation.

280. Managing durable resistance to stem canker of oilseed rape: molecular tools and mathematical modelling . Neal Evans (1), Thierry Rouxel (2), Hortense Brun (3), Peter Gladders (4), Xavier Pinochet (5), Malgorzata Jedryczka (6), Ingrid Happstadius (7), Michel Renard (8). (1) Rothamsted Research, Harpenden, UK (2) INRA, PMDV, Versailles, France (3) INRA UMR BiO3P, Le Rheu, France (4) ADAS Boxworth, Cambridge, UK (5) CETIOM, Thiverval-Grignon, France. (6) Institute of Plant Genetics PAS, Poznan, Poland. (7) Svalöf Weibull AB, Svalöv, Sweden. (8) UMR APBV, Le Rheu, France

Stem canker (blackleg) of crucifers, caused by *Leptosphaeria maculans*, is a damaging disease of oilseed rape worldwide and is responsible for substantial annual crop losses in Europe. In 2003, the European Commission funded the SECURE multinational project (StEm Canker of oilseed rape: molecular tools and mathematical modelling to deploy dUrable REsistance: QLK5-CT-2002-01813) which aims to deliver a model for deployment of cultivars with durable resistance. Project work includes the development of a *L. maculans* life-cycle model, genomic analysis and fitness studies using virulent and avirulent races of the pathogen, studies of the effects of plant genetic background and environmental factors on durability of resistance under controlled conditions and at field sites across Europe. Data generated during the project will be used to develop a mathematical model that can be used to produce recommendations for the sustainable deployment of durable resistance against stem canker of oilseed rape.

281. Comparative genomics and synteny studies revealing the reservoir of secreted proteins in *Phytophthora*. Rays H.Y. Jiang, Brett Tyler* and Francine Govers. Plant Sciences Group, Laboratory of Phytopathology, Wageningen University, and Graduate School Experimental Plant Sciences, The Netherlands.* Virginia Bioinformatics Institute, Virginia Polytechnic and State University, Blacksburg VA, USA

Pathogenic fungi and oomycetes possess a wide range of molecules to interact with their hosts. Proteins secreted by plant pathogens are of ultimate interest because these proteins might be effector molecules that play important roles in pathogenesis. The presence of signal peptides and transmembrane domains was analyzed on all annotated genes in two sequenced *Phytophthora* genomes revealing the whole reservoir of secreted proteins. A total of 1570 and 1256 putative secreted protein genes from *P. sojae* and *P. ramorum*, respectively, were investigated for their sequence diversity, expansion of family members and genome organization. More than 80% of the secreted protein genes form gene families, and many of the families are clustered in the genome. Differences in expansion of gene families in different *Phytophthora* spp. were observed, and these expansion patterns may explain the difference in their pathogenicity. Some genes are located in genomic regions having many re-arrangements and insertions/deletions and these "hotspots" are particular interesting to explore.

282. Developing a lichen model system. Suzanne Joneson[1] and François Lutzoni[1]. [1]Duke University, Durham NC, USA.

Lichens are the mutualistic association of a fungus and an alga. This successful and terrestrially ubiquitous lifestyle is shared by 40% of all higher fungi. Multiple origins of lichen forming fungi share a common ancestor with pathogenic fungi. Both types of fungi make contact with bacterial, plant (including green algae) and/or animal cell walls, and must be able to recognize foreign cells as acceptable or unacceptable symbionts. With this as our primary hypothesis, we would like to explore to what extent lichen-forming fungi share common recognition, and cell signaling pathways with other mutualistic fungi. Using *Cladonia grayi*, we are developing a system in which we can detect differential gene expression between the symbiotic and aposymbiotic states, identifying candidate genes through homology searches. Additionally we are trying to develop a transformational system in which we can make mutants and ultimately targeted transformants. Our current goal of developing a model lichen-forming fungus will lay the groundwork for future dissection of the fungal-algal symbiosis, as well as the evolution of varying symbioses from a common ancestor.

283. Elucidating the Role of the F-box Protein Frp-1 in Pathogenesis of Fusarium oxysporum. W. Jonkers, R. G. E. Duyvesteijn, B. J. C. Cornelissen and M. Rep. University of Amsterdam, Swammerdam Institute for Life Sciences, Plant Pathology, Amsterdam, the Netherlands.

During evolution, plants have developed effective ways to defend themselves against microbial invasion. A microbial pathogen has to break through these defences in order to colonize the plant. To investigate the genetic basis of this ability of pathogens, we use the interaction between the soil-borne fungus *Fusarium oxysporum* f.sp. *lycopersici* and its host tomato as a model system. Using insertional mutagenesis of an F-box protein called Frp1 was found to be required for pathogenicity. It was shown that this protein, like other F-box proteins, binds to Skp1, a subunit of the E3 complex. This complex is involved in the ubiquitination of proteins recruited by F-box proteins.
One approach towards determination of the function of Frp1 is to find interacting proteins. To do so, a yeast two-hybrid screening using $Frp1^{L226S}$ will be carried out. This Frp1 mutant has lost the ability to bind Skp1, but is still able to bind other interactors. For yeast two-hybrid screening, a genomic *Fol* Y2H library is being constructed.
The second approach to determine the function of Frp1 is to study the phenotype of the mutant. Microscopic studies of GFP-labelled wild type *Fol* and an *?frp1* mutant showed that the mutant has lost its ability to colonise the roots. The mutant also showed less growth on agar plates with alcohol, root-exudate sugars, organic acids or cell wall components as the sole carbon source. Probably, the mutant has a defect in assimilation of certain carbon sources which might be related to loss of pathogenicity. To investigate this further, transcript levels of genes involved in carbon source assimilation will be determined.

284. Regulatory cascades during pathogenic development of *Ustilago maydis*. J. Kämper, M. Scherer, M. Vranes, C. Pothiratana. MPI for terrestrial Microbiology, Marburg, Germany

In the plant pathogen *Ustilago maydis*, the change from saprophytic growth to the biotrophic stage is controlled by a unique genetic switch, namely the bE and bW homeodomain proteins encoded by the *b*-mating type locus. Our aim is to understand the processes that lead to the establishment of the biotrophic stage. To this end, we have employed genome-wide DNA arrays for *U. maydis*. The arrays were used to depict the gene expression profiles of *U. maydis* cells in response to the activation of the bE/bW heterodimer in axenic culture, but also at early and late plant infection stages. For detailed analysis of the differentially regulated genes, we have focused so far on genes encoding proteins with potential regulatory functions. By this means we were able to identify three novel pathogenicity factors for *U. maydis*. The expression profiles and phenotypes of the respective mutants revealed that they are part of a network regulating pathogenicity and filamentous growth. One of the genes, *rbf1*, encodes a zink finger transcription factor that is required (and sufficient) for the expression of the majority of *b*-regulated genes, by that assigning a central role within the network. Among the *rbf1* dependent genes are *hdp1* and *riz1*, encoding a homeobox protein and a potential zink finger transcription factor. While *hdp1* can be linked to filamentation, *riz1* is required during the early infection stages.

285. Signalling cascades in *Ustilago maydis* : The use of functional genomics for the identification of downstream genes. R. Kahmann, T. Brefort, H. Eichhorn, F. Lessing, B. Winterberg, P. Mueller and A. Mendoza, Max Planck Institute for terrestrial Microbiology, Marburg, Germany

Ustilago maydis is a dimorphic fungus that switches from a yeast-like haploid stage to a filamentous dikaryon after mating. In nature it is the dikaryon that is able to differentiate infection structures and cause disease on corn plants. In this system cAMP signaling as well as two MAP kinase modules regulate discrete steps during pathogenesis. To identify downstream targets of these pathways we have applied molecular tools for functional genome analysis. In particular we have constructed strains where the individual pathways can be genetically activated and have followed changes in gene expression pattern using Affymetrix arrays. This has allowed us isolate novel genes whose products play crucial roles during pathogenesis. I will describe the identification of gene clusters involved in iron uptake and demonstrate that the reductive iron uptake system affects virulence. In addition, I will describe a novel HMG-domain transcriptional regulator of *prf1* and how it fits into the regulatory network controlling signalling.

286. Molecular and Cellular Biology of Biotrophic Interactions in Rice Blast Disease. Prasanna Kankanala and Barbara Valent. Department of Plant Pathology, Kansas State University, Manhattan, KS 66503 USA.

The filamentous ascomycete fungus *Magnaporthe grisea* is the hemi-biotrophic pathogen that causes rice blast disease. We hypothesize that the fungal effectors play a dual role in establishing biotrophy and in R-gene mediated resistance. To identify candidate fungal effectors we are using the Zeiss Positioning Ablation Laser capture Microdissection (PALM) system to collect biotrophic invasive hyphae from the infected tissues. RNA extracted from these samples will be used to do microarray experiments and analyze the gene expression patterns in both resistant and susceptible interactions. To study the cellular changes during the biotrophic infection process we developed a transgenic fungal strain expressing EYFP constitutively under control of the fungal ribosomal promoter RP27. The initial plasmolysis experiments indicate that the host membrane is intact when the fungus grows inside the plant cell. To study the host membrane-pathogen interface in the first invaded plant cell, we are doing live-cell confocal microscopy with the FM4-64 fluorescent endocytotic tracer that labels the membranes. Our preliminary imaging indicates that the invasive fungal hyphae inside the host cells are surrounded by a very prominent membrane. Experiments are underway to determine the source and nature of this membrane.

287. The role of *Botrytis cinerea* endopolygalacturonases in pathogenesis: BcPG2 is the most important virulence factor. Ilona Kars, Lia Wagemakers, Geja Krooshof, Rob Joosten, Jac Benen and Jan van Kan. Lab. of Phytopathology, Wageningen University, The Netherlands

During infection of its host plants, *B. cinerea* secretes a considerable number of cell wall-degrading enzymes, among which are six polygalacturonases and two pectin methylesterases. The presence of multiple genes encoding CWDEs raises numerous questions about the exact function of such a set of enzymes. Several approaches were taken to understand how these pectinases are involved.
Mutants were generated in several BcPG and BcPME encoding genes by targeted mutagenesis. Virulence assays showed that elimination of *Bcpg2* caused a reduction of virulence on different hosts, whereas elimination of *Bcpg3*, *Bcpg5*, *Bcpme1* and *Bcpme2* did not have an effect on the virulence of strain B05.10.
Five BcPGs were produced in *P. pastoris*, purified and characterized. Infiltration of BcPGs into plant tissue caused different symptoms. Especially BcPG2 was extremely potent in causing necrosis in a range of host plants. Other isozymes did not cause such severe symptoms. Symptoms differed per enzyme and per plant species. In broad bean BcPG2 very rapidly (To distinguish cell death by maceration and plasmolysis from cell death by protein recognition, we conducted two types of experiments: 1) infiltration of inactive mutant protein (mutated in active site) produced in *P. pastoris* and 2) *A. tumefaciens*-mediated transient expression of active as well as inactive BcPGs. Results of this thesis work will be presented.

288. Characterization of the promoter region of hypoviral regulated fungal hydrophobin gene from *Cryphonectria parasitica*. Myoung-Ju Kim, Min-Jae Kim, Seung-Moon Park, Moon-Sik Yang and Dae-Hyuk Kim. Division of Biological Sciences, Basic Science Research Institute, Chonbuk National University, Dukjindong 664-14, Jeonju, Chonbuk 561-756, Korea

Cryparin is a cell wall associated fungal hydrophobin of chestnut blight fungus, *Cryphonectria parasitica*. Although the cryparin is encoded by a single copy gene (*crp*1), its expression is so strong that the amount of transcript can reach up to 22% of total mRNA. In addition, it is one of well-known examples of the transcriptional down-regulation of a fungal gene by hypovirus. To identify regulatory elements in *crp*1 promoter, transcriptional fusions of EGFP to various lengths of *crp*1 promoter were used to transform *C. parasitica* and EGFP activity of resulting transformants was compared in the presence and absence of hypovirus. Deletion of the promoter region between -1280 to -907 resulted in a drastic decrease of the promoter activity and the presence of corresponding fragment was required for the hypovirus-mediated down-regulation, which suggests that the 376 bp region is necessary for the high level expression of cryparin and hypoviral regulation. The mobility shift assay using the corresponding 367 bp region revealed the presence of cellular factor(s) of *C. parasitica* suggesting that it contains the cis-acting regulatory elements involved in the cryparin expression and hypovirus regulation.

289. Lipid-induced filamentation in *Ustilago maydis*. J. Klose and J.W. Kronstad. The Michael Smith Laboratories, Dept. of Microbiology and Immunology, The University of British Columbia, Vancouver, BC, Canada

The transition from a yeast-like to a filamentous morphology in *Ustilago maydis* is regulated by conserved cAMP and MAPK signaling pathways and is correlated with virulence because only the filamentous cells can invade host plant and cause disease. We showed recently that lipids induce filamentation in *U. maydis* (Mol. Microbiol. 2004. 52:823-835). This may be relevant to infection of the plant because the components of the signaling networks are required for the dimorphic transition in response to lipids, and the morphological features of the lipid-induced filaments formed *in vitro* resemble those observed *in planta*. We are exploring the potential metabolic and signaling roles of lipids to further characterize the morphological transition. On the metabolic side, we have disrupted the mfe2 gene encoding the multifunctional enzyme involved in beta-oxidation of fatty acids. The strains defective in mfe2 did not grow on medium with oleic acid as the sole carbon source. Preliminary results indicate that the mutants respond morphologically to saturated but not to unsaturated fatty acids. The observations suggest possibility to separate utilization of fatty acids as a carbon source from the filamentation response and perhaps the saturation state of fatty acids may influence the dimorphic switch. The mfe2 gene was disrupted in strains of compatible mating type and the resulting mutants were able to mate. Subsequent inoculation of the compatible mutants into corn seedlings revealed that they were attenuated for virulence although weak disease symptoms were observed. The defect in virulence may result from a metabolic deficiency that prevents proper utilization of host nutrients and blocks extensive proliferation. Additional characterization of the mfe2 mutants is underway including examination of the fungal morphology during growth *in planta*.

290. Withdrawn.

291. Pathogenicity-specific domains in Ubc2, a pheromone-responsive adapter protein. Steven J. Klosterman, Alfredo D. Martinez-Espinoza, Jeffrey R. Seay, David L. Andrews, Scott E. Gold. Plant Pathology, University of Georgia, Athens.

The plant pathogenic fungus *Ustilago maydis* alternates between a haploid budding form and a dikaryotic filamentous form. Genes involved in the cAMP and MAPK pathways that control mating and morphogenesis have been identified previously by complementation of mutants that suppress a constitutively filamentous *uac1* (*Ustilago* adenylate cyclase) haploid mutant. These genes were named *ubc* for *Ustilago* bypass of cyclase. The protein encoded by *ubc2* is essential for virulence and possesses distinct protein interaction domains (N-terminal SAM, RA and two C-terminal SH3 domains). Ubc2, like other basidiomycete database orthologs, has two C-terminal SH3 domains while related ascomycete proteins are truncated and lack this region. Site-directed mutagenesis indicated that the N-terminal SAM and RA domains are necessary for mating and filamentous growth. Interestingly, C-terminal deletion mutants lacking the SH3 domains are still capable of mating and filamentous growth like the wild type but are nonpathogenic. The C-terminal domains of Ubc2 thus act as a basidiomycete-specific pathogenicity determinant. Yeast two-hybrid screens identified a number of proteins interacting with the various Ubc2 domains, including SAM-SAM heterodimer formation between Ubc2 and the pheromone-responsive Ubc4/Kpp4 MAPKK kinase. The other candidate proteins identified will be discussed.

292. Differentially expressed genes and host specifity of ectomycorrhizal fungi genus Tricholoma. Katrin Krause and Erika Kothe. Friedrich Schiller university, Jena Germany

RNA fingerprinting was carried out to identify genes showing differential expression in ectomycorrhiza between basidiomycete *Tricholoma vaccinum* and compatible host spruce *Picea abies* using ectomycorrhizal roots of different stages, pure roots and cultures of the fungus. Resulting PCR fragments were verified and the clones origin and expression pattern were checked. Of the fungal fragments with mycorrhiza-specific expression sequence analyses were performed to identify the nature of the encoded protein. Among them were genes with function in plant pathogen response, signal transduction, nutrient exchange, growth in plant and stress answer. Two genes an aldehyde dehydrogenase and a retrotransposon were investigated more intensively. Expression studies were performed with quantitative RT-PCR testing fungal mRNA of several aldehyde dehydrogenase substrates containing media, of stress inducible conditions, of different mycorrhizal stages, tissues and low compatible symbiotic interaction with pine *Pinus sylvestris*. The spreeding of the retrotransposon in genus *Tricholoma* was examined in 10 *Tricholoma* species also using ITS sequences, because it has a bit higher amino acid identity to RTase and RNase domains of retrotransposons of phytopathogenic ascomycetes than to MarYI of *Tricholoma matsutake*.

293. Identification and mapping of *Pyrenophora teres* f. *teres* genes conferring avirulence on barley. Lai, Z., Steffenson, B.J., Faris, J.D., Cartwright, R.D., Webster, R.K., Weiland, J.J., and Friesen, T.L. Plant Pathology, North Dakota State University

Pyrenophora teres f. *teres* is an ascomycetous fungus that causes net blotch of barley, a serious foliar disease throughout the world. A *P. teres* f. *teres* cross between parental isolates 0-1 and 15A yielded 78 single ascospore progeny. A molecular map consisting of 108 AFLP markers and 15 linkage groups was constructed. The linkage groups spanned a total genetic distance of approximately 909 cM. Electrophoretic karyotype analysis revealed a minimum of six chromosomes ranging from 1.8 Mb to 6.0 Mb with a minimum estimated genome size of 23 Mb. Net blotch infection response data were generated for this population by performing conidial inoculations of each progeny isolate on the four barley lines Canadian Lake Shore, Ming, Tifang, and Prato, which showed a differential response to parental isolates. The molecular map was used in conjunction with the phenotypic data to identify linkage groups containing loci associated with avirulence. Phenotype data from each of the four barley lines indicate that avirulence is controlled by major genes in this population and that these avirulence/virulence genes are linked.

294. Fungal tetraspanins: key players in host plant invasion. Lambou K., Fargeix C., Catusse J., Gourgues M., Cottier F. and Lebrun M.H.. FRE2579 CNRS / Bayer cropscience Physiologie des plantes et des champignons. 14-20 rue Pierre Baizet 69263 Lyon cedex 09 (France)

The non-pathogenic mutant punchless from the rice blast fungus *Magnaporthe grisea* is unable to invade its host leaves. This invasion process requires the differentiation of a fungal cell, the appressorium, specialized in the penetration of the pathogen into host leaves. The mutant punchless differentiates appressoria that are non-functional, as they cannot direct the penetration of the fungus into host leaves. The gene inactivated in this mutant encodes a putative integral membrane protein of 225 AA (Pls1) that exhibits classical features of animal tetraspanins. Genes orthologous to PLS1 were identified in other fungi such as *Botrytis cinerea, Colletotrichum lindemuthianum, Fusarium graminearum* and *Neurospora crassa* defining a new tetraspanin family with highly conserved domains. Deletion of PLS1 orthologs in the plant pathogenic fungi *B. cinerea* (C. Levis, A. Simon, PMDV, INRA, France) and *C. lindemuthianum* (C. Veneault, D. Parisot, R. Lauge, T. Langin, IBP, CNRS-UPS-INRA, France) leads to non-pathogenic mutants. These mutants have the same defect as *M. grisea* PLS1 deletion mutant, as they differentiate appressoria unable to direct fungal penetration into host plants. These results suggest that fungal tetraspanins control a conserved appressorial function essential for the penetration of fungi into host leaves. Amino acids from domains conserved among fungal tetraspanins were modified by site-directed mutagenesis in *M. grisea* Pls1. The functionality of these mutant proteins was assessed by complementation of the *M. grisea* non-pathogenic PLS1 deletion mutant. The four cysteines from Pls1 ECL2 and the C-terminus tail are required for Pls1 function. Pls1 is only expressed in appressoria and its Pls1-GFP fusion protein is mainly localized in the vacuoles of this fungal cell. Comparison of genome wide expression profiles of wild type and PLS1 deletion mutant appressoria are currently used to identify the downstream targets of Pls1.

295. Involvement of autophagy in the appressorium functionality of *Colletotrichum lindemuthianum*. Eugénie Bard, Richard Laugé et Thierry Langin. Phytopathologie Moléculaire, Institut de Biotechnologie des Plantes (UMR-CNRS 8618), Bât 630, Université Paris-Sud, 91405, Orsay, France.

Colletotrichum lindemuthianum is the causal agent of the anthracnose disease on common bean, *Phaseolus vulgaris*. This fungus attacks all green parts of the plant ending in necrosis of infected tissues. Previous analyses of non pathogenic mutants obtained through insertional mutagenesis allowed the identification of a mutant that differentiates mature appressoria but is unable to penetrate the plant tissue. A mutation in the clk1 gene (Dufresne et al., MPMI (1998), 11, 99-108), that encodes a Sérine/Threonine kinase, is responsible for the observed phenotype. clk1 is the orthologous gene of the Saccharomyces cerevisiae atg1 gene, that triggers the autophagy process under starvation conditions. Functional homology between clk1 and atg1 was demonstrated via the complementation of yeast atg1- mutants. In order to demonstrate the involvement of autophagy in the functionality of C. lindemuthianum appressoria, clapg2 (C. lindemuthianum autophagy-2) the orthologue of the yeast atg8 gene, that is compulsory for the formation of the yeast autophagosomes, was cloned. The clapg2 gene is induced in vitro under nitrogen starvation as expected. Furthermore, upon plant inoculation, clapg2 is also transiently induced during appressoria development. Taken together, these results suggest a role for autophagy in the mobilization of cell ressources for appressorium functionality. Nul mutants (clapg2::hph) are currently under construction to validate this hypothesis.

296. Transcriptional regulation during the infection process of *Colletotrichum lindemuthianum*. Marcos Soares, Eugénie Bard, Anne-Laure Pellier, Richard Laugé et Thierry Langin. Phytopathologie Moléculaire, Institut de Biotechnologie des Plantes (UMR-CNRS 8618), Bât 630, Université Paris-Sud, 91405, Orsay, France.

Colletotrichum lindemuthianum is a hemibiotrophic fungus that causes anthracnose disease on common bean. It develops along its infection cycle a strict succession of specialized cell structures: appressorium, infection vesicle, primary hyphae, and secondary hyphae that coincidate with the succession of the penetration, biotrophy and necrotrophy phases. Such a life cycle certainly requires precise genetic programs to be fulfilled in order to accomplish developmental and metabolic modifications/adjustments. Two alternative strategies are developed in order to identify transcriptional activators involved in the control of this complex process. The molecular analysis of non-pathogenic *C. lindemuthianum* mutants led to the identification of clta1 a Zn finger transcriptional activator that controls the transition between biotrophy and necrotrophy (Dufresne et al., Plant Cell, 2000). Putative targets giving clues about its involvement in development (hyphal transition) and/or metabolism (shift of trophic mode) are currently searched directly by substractive hybridization. clnr1, the areA/NIT-2 orthologue, was cloned and demonstrated to be the global nitrogen regulator of C. lindemuthianum. clnr1- mutants are non pathogenic but surprisingly impaired at the beginning of necrotrophy (Pellier et al., Molecular Microbiology, 2003). We have also cloned the orthologue of the pacC gene. Its role in vitro is currently studied, and the construction of null mutants is on the way in order to evaluate the role of pH regulation during the infection. In parallel, an EST project provided us with many genes exhibiting typical domains of transcriptional activators. We plan to test more broadly a number of them for involvement in the infection process of the fungus.

297. Virulence gene discovery in the Brassica pathogen, *Alternaria brassicicola* using functional genomics. Yangrae Cho [1], Josh Davis [1], Carlos Mauricio La Rota [1], Robert Cramer[2], Christopher Lawrence[1]. [1] Virginia Bioinformatics Institute, Blacksburg, VA. [2] Duke University Medical Center, Durham, NC.

Mitogen-activated protein (MAP) kinases have been shown to be involved in signal transduction cascades required for virulence in several pathogenic fungi. We cloned the yeast fus3 homolog (AMK1) in *A. brassicicola*, a necrotrophic fungal pathogen of cultivated Brassicas. Knock out (KO) mutants showed a complete loss of pathogenicity on three Brassica species and two Arabidopsis ecotypes. The mutants demonstrated partially restored pathogenicity, as determined by measuring lesion diameters, compared to the wild type (WT) fungus only when leaves were wounded prior to inoculation. Transcription patterns were compared between WT and mutants for seven genes encoding secreted hydrolytic enzymes and three putative toxic proteins using semi-quantitative RT-PCR. Mutants showed elevated expression of many of the secreted hydrolytic enzyme and toxin genes compared to WT on wounded leaves, but were expressed at low levels compared to WT on intact leaves. This suggests that signals derived from wounded tissues may activate downstream components of the pathway with important roles in pathogenicity. To find additional infection-related fungal genes, ESTs from a full-length cDNA library constructed from RNA isolated from infected rapeseed were characterized. We found numerous candidate pathogenicity genes from sequencing over 3,000 ESTs thus far. We have KOÕd three secreted hydrolytic enzyme genes, one zinc finger transcription factor, and two signaling pathway genes and observed a reduction in pathogenicity in several cases. We will present gene annotation of sequences derived from the infected rapeseed library and phenotype information for each of the six initial KO mutants.

298. Two putative isocitrate lyase genes (*GzICL1* and *GzICL2*) are required for virulence and sexual development in *Gibberella zeae*. Seung-Ho Lee[1], Sung-Hwan Yun[2], and Yin-Won Lee[1]. [1]School of Agricultural Biotechnology, Seoul National University, Seoul 151-921; [2]Division of Life Sciences, Soonchunhyang University, Asan, 336-745, Korea

Isocitrate lyase (ICL) is one of two enzymes consisting of the glyoxylate pathway that are involved in the metabolism of two-carbon compounds such as acetate. Recent studies on *Leptosphaeria maculans* and *Magnaporthe grisea* revealed that the *ICL* genes were essential for disease development by these phytopathogenic fungi. To elucidate the function of ICL in the cereal head blight fungus *Gibberella zeae*, we identified two orthologs of the *ICL* gene, designated *GzICL1* and *GzICL2* from the *G. zeae* genome database. Transgenic strains of *G. zeae* deleted for either of two *GzICL* (designated *delGzICL1* and *delGzICL2*, respectively), or for both (*delGzdIl*) were generated using a gene replacement strategy. Transgenic *delGzICL1* strains were normal compared with its wild-type progenitor except ascospore formation; they produced fewer perithecia, when selfed. In contrast, *delGzICL2* produced fertile perithecia as many as wild-type, but were slower in hyphal growth on medium containing 0.25% glucose or C_{12} fatty acid (0.25% Tween 20). For virulence on barley heads, both *delGzICL1* and *delGzICL2* caused disease symptoms as severe as wild-type. Interestingly, the *delGzdIl* mutants showed significantly reduced virulence on host plant; they produced no perithecia on mating plates. These results strongly suggest that both *GzICL1* and *GzICL2* genes are required for virulence as well as sexual development in *G. zeae*.

299. Fungal peroxisomes in the *Stagonospora nodorum* / wheat interaction. Robert Lee, Kasia Rybak, Peter Solomon and Richard Oliver. Australian Centre for Necrotrophic Fungal Pathogens, Murdoch University, Perth, WA, Australia.

Peroxisomes contain biochemical pathways that are important for the growth and development of fungi. Enzymes for methanol oxidation and peroxide dissimilation, the fatty acid beta-oxidation pathway and the glyoxylate cycle are located in peroxisomes. Peroxisomes have been recently reported to be essential for appressorium development and spore germination in fungal phytopathogens and this paper describes our recent work to further investigate the roles of peroxisomes in fungal pathogenesis. A *S. nodorum* strain with peroxisomally-targeted GFP was prepared by transformation with a GFP expression construct encoding a COOH-terminal peroxisomal targeting signal. Peroxisome proliferation and increased GFP fluorescence were observed with addition of methanol or oleate to culture media and peroxisomes were observed throughout surface hyphae in infected wheat leaves but not in hyphae growing inside leaf tissue. GFP-containing peroxisomes in a malate synthase-deficient mutant (*mls1*-8) exhibited a 30-fold increase in fluorescence. A functional analysis study of genes encoding peroxisomal proteins is also underway. A cDNA library prepared from oleic acid-grown *S. nodorum* was screened with a SSH probe enriched in genes expressed in oleate media. Several genes that encode enzymes from known pathways were identified, along with others that may have novel functions in fungal peroxisomes. Progress in the characterization of a number of these genes will be discussed.

300. QTLs for pathogenicity on *Pinus silvestris* located in a *Heterobasidion annosum* genetic linkage map. Mårten Lind, Åke Olson and Jan Stenlid. Swedish University of Agricultural Sciences, Uppsala, Sweden

The basidiomycete *Heterobasidion annosum* is the most devastating fungal pathogen on conifers in the world. Its intersterility groups S and P are named after host preference (spruce and pine). Using a mapping population of 102 single spore isolates, originating from a compatible mating between North American isolates of the P and S groups, a genetic linkage map of the *Heterobasidion annosum* genome was constructed. The map consists of 39 linkage groups and spans 2252 cM in total. The average distance between two markers is 6.0 cM.
In order to map QTLs for pathogenicity, two methods were used. First, 29 two weeks old *Pinus silvestris* seedlings were grown in homogenized mycelia for 25 days. Every third day the number of dead seedlings were estimated. The virulence was determined as the regression value of the disease increase rate for each isolate. The data suggested a QTL on linkage group 11 with a LOD of 3.09, explaining 16.4% of the variation in virulence.
Second, ten plants of one year old *P. silvestris* was infected with a fungal infested wooden plug in a wound in the cambium. After four weeks the necrosis was measured upstem and downstem from the cambial wound. The virulence was determined as mean necrosis length for each isolate. The data suggested one QTL on linkage groups 15 and one on group 20, with peak LOD values of 3.29 and 4.24, explaining 15.8% and 18.2% of the variation in virulence, respectively.
These QTLs will be identified and characterised in future studies.
This project is funded by The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning, FORMAS.

301. The role of alternative carbon metabolism in *Candida*-phagocyte interactions. M. Ramirez[1], J. Bender[2], G. Fink[2], and M. Lorenz[1]. [1] Dept. of Microbiology, Univ. of Texas Health Science Center, Houston [2] Whitehead Inst., Cambridge, MA

Candida albicans occupies a unique niche as both a ubiquitous commensal and common pathogen of humans. Because the most serious infections result from defects in innate immunity, we have studied the interaction between *C. albicans* and phagocytes using genomic microarrays. Phagocytosis of *C. albicans* by murine macrophages stimulates a massive response involving nearly 10% of the genome, including altered carbon utilization, translation repression, and stress responses. We are focusing on the role of alternative carbon utilization and have found that the glyoxylate cycle, used to assimilate simple carbon compounds like acetate, is upregulated upon phagocytosis and that it is required for full virulence in a mouse model. Preliminary evidence suggests that fatty acid beta-oxidation (the product of which enters the glyoxylate cycle) is absolutely required in vivo as *fox2* mutants are completely avirulent. In addition, the regulation of beta-oxidation and the glyoxylate cycle appears to be linked in *C. albicans*, unlike in *S. cerevisiae*, suggesting a tight functional link in the pathogen. The importance of this pathway is further reinforced by the induction by macrophage contact of several genes implicated in acetate homeostasis. Taken together, these results indicate that the ability to recognize and adapt to carbon sources that are poor in both quantity and quality is a key component of the in vivo success of *C. albicans*.

302. Functional complementation and comparative expression analysis of CPS1, a common virulence determinant in filamentous ascomycetes. Shun-Wen Lu and B. Gillian Turgeon, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Ascomycetes share a highly conserved acyl-AMP ligase-like protein (CPS1), originally identified in the maize pathogen, *Cochliobolus heterostrophus* (*Chet*). We have previously demonstrated that CPS1 is required by diverse plant pathogens for virulence on their hosts. Towards further understanding of the biological and biochemical nature of CPS1, we have: 1) Complemented a reduced virulence, *Chet CPS1*-deletion strain (delta*Chcps1*), with a cloned native *ChCPS1* gene. 2) Expressed *ChCPS1* orthologs from the wheat scab fungus, *Gibberella zeae* (*GzCPS1*) and the saprobe, *Neurospora crassa* (*NcCPS1*) in delta*Chcps1*. 3) Examined *ChCPS1, GzCPS1*, and *NcCPS1 in vitro* expression patterns. Preliminary results indicate that: 1) The native *ChCPS1*, and the heterologous *GzCPS1* and *NcCPS1* genes all restore wild-type virulence capability to the delta*Chcps1* strain. 2) *CPS1* is expressed in a species- and culture condition-dependent manner, and there appears to be more than one type of transcript. One transcript is expressed differently in the pathogen *G. zeae* compared to the closely related saprobe, *N. crassa*. These results suggest that CPS1 function is likely the same in saprobes and pathogens and that this function is also required for the pathogenicity niche. Although the CPS1-mediated virulence mechanism is unclear, morphological abnormalities, observed when *cps1*$^{-}$ mutants of *C. heterostrophus* colonize the host, and *CPS1* expression data suggest that it might be important for adaptation to stress conditions, including those derived from host defense during fungal infection.

303. Ptr ToxA: Hitting where it hurts. Viola A. Manning[1], Ganapathy N. Sarma[2], P. Andrew Karplus[2], Lynda M. Ciuffetti[1]. [1]Department of Botany and Plant Pathology, [2]Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR, USA.

Ptr ToxA, the first proteinaceous, host-selective toxin isolated, is produced by the fungus *Pyrenophora tritici-repentis* and induces necrosis in sensitive but not insensitive wheat cultivars. Understanding the site-of-action of ToxA and how its structure impacts symptom development will provide insight to formulating controls for tan spot of wheat. Several lines of evidence including immunolocalization, GFP-ToxA localization and immunoprecipitation suggest that the difference between ToxA-insensitive and -sensitive cultivars lies in the ability of the toxin to be internalized. Results suggest that toxin is internalized only in sensitive cultivars. Biolistics indicate that if toxin could be internalized in insensitive wheat, cell death would result. ToxA appears to localize to the cytoplasm and chloroplast following internalization. A single clone isolated from a yeast two-hybrid screen of a library produced from a ToxA-sensitive wheat cultivar supports chloroplast localization. Crystal structure analysis of ToxA has confirmed a putative protein-protein interaction interface, a vitronectin-like sequence that contains an RGD cell attachment motif, previously identified via site-directed mutagenesis. Whether this domain is necessary for internalization, for protein-protein interactions after toxin enters the cell, or both, is currently under investigation.

304. Chromosomal location and expression of *ftf1* and *ftf2* genes in pathogenic strains of *F. oxysporum*. N. Martín-Rodrigues, B. Núñez-Corcuera, B. Ramos, M.A. García-Sánchez, , A.P. Eslava and J.M. Díaz-Mínguez. Área de Genética. Centro Hispano-Luso de investigaciones Agrarias (CIALE). Universidad de Salamanca. 37007, Salamanca, Spain.

We have described two novel *Fusarium* transcription factors: *ftf1* and *ftf2*, wich show a high nucleotide and aminoacidic homology but very different genomic architecture and expression. Both genes contain an ORF of 3240 nt including a 49 bp intron. The predicted 1080 amino acid protein contains a Zn(II)-Cys6 binuclear cluster DNA-binding motif with homology to various fungal regulatory proteins. The gene *ftf1* is specifically expressed on early stages of infection, while *ftf2* shows a very low level of expression in vivo and in vitro.
We have analyzed the presence and expression of both genes in 14 formae speciales of *F. oxysporum*. The results mostly confirm those previously obtained in *F. oxysporum* f.sp. *phaseoli*. Chromosome hibridizations show the presence of *ftf2* in all the formae speciales and several copies of *ftf1* in different chromosomes. We have also analyzed the presence of transposon marsu and the homologue of *bimB3* (*Aspergillus nidulans*). RT-PCR analyses of expression using primers specific for each gene show that *ftf2* is expressed in vitro in all the formae speciales.

305. Spatial and Temporal *In Planta* Expression of a Bioprotective Metabolite Gene from *Epichloë festucae* using the GUS Reporter Gene. Kimberley May, Michelle Bryant, Xiuwen Zhang, Barbara Ambrose and Barry Scott. Center for Functional Genomics, IMBS, Massey University, Palmerston North, New Zealand

Fungal endophytic species of the genera *Epichloë* and *Neotyphodium* form mutualistic symbioses with cool season grasses of the family Pooideae. Isolates of *E. festucae* and *N. lolii* provide protection to their host against grazing animals through the production of lolitrem alkaloids. A cluster of genes involved in the biosynthesis of lolitrems (*ltm* genes) have been isolated and characterised from *N. lolii* and *E. festucae*. To gain an understanding of where and when these genes are expressed *in planta*, several P*ltmM-uidA* constructs were prepared and transformed into *E. festucae*. Associations between perennial ryegrass (*Lolium perenne*) and these transformants were established and GUS expression analysed. Expression of GUS was observed in vegetative tillers but not in the roots. In the spikelets, expression was initially observed in the rachis, glumes, lemma, palea and rachilla but not in the male or female reproductive organs. However, in later stages, GUS expression appeared to occur largely in the female reproductive organs with little expression observed in the lemma, palea or the male reproductive organs. Future work will involve an analysis of GUS expression patterns in germinating seed and young seedlings.

306. Genetic and functional analyses of a 96 kb region harboring a putative avirulence locus from the fungal wheat pathogen *Mycosphaerella graminicola*. Rahim Mehrabi, Odette Mendes, Cees Waalwijk, Theo van der Lee and Gert H. J. Kema. Wageningen University and Research Centre, Plant Research International B.V., P. O. Box 16, 6700 AA Wageningen, The Netherlands.

Segregation analyses in a mapping population of a cross between two Dutch field isolates, IPO323 (avirulent) and IPO94269 (virulent) of the wheat pathogen *Mycosphaerella graminicola* demonstrated that avirulence in IPO323 is controlled by a single locus. We generated BAC libraries from both isolates and constructed BAC contigs of each isolate that include all markers cosegregating with avirulence. Sequence analysis of this 96 kb region predicted over 100 ORFs from which we selected two candidate avirulence genes for further analyses. The first candidate, designated ORF43, was a small cystine rich gene expressed in planta, with no homology to the public DNA databases and was partly deleted in the virulent parent. Targeted disruption of ORF43 in the avirulent isolate did not affect the phenotype indicating this gene is not an avirulence factor. The second candidate is located about 10 kb downstream of ORF43 and has strong homology to polyketide synthases. Disruption of this gene in both isolates did not affect the phenotype indicating that this gene is neither an avirulence nor a virulence factor. To identify the avirulence gene in this region we are currently swapping larger BAC derived DNA fragments between both IPO323 and IPO94269 and expect to be more successful with this non biased approach.

307. *Mlt2*, a MAP kinase of *Mycosphaerella graminicola* involved in cell wall integrity, is dispensable for penetration but is essential for invasive growth. Rahim Mehrabi, Cees Waalwijk, Theo van der Lee, and Gert H. J. Kema. Wageningen University and Research Centre, Plant Research International B.V., P. O. Box 16, 6700 AA Wageningen, The Netherlands.

Through analyses of EST libraries of *Mycosphaerella graminicola*, we identified a full-length cDNA clone with high homology to a mitogen-activated protein (MAP kinase), *Slt2* in *Saccharomyces cerevisiae*. This MAP kinase, designated *mlt2*, possesses a 1242 bp open reading frame, and encodes a 414aa protein. We generated knock-outs of this gene and identified several altered phenotypes *in vitro* as well as *in planta*. On yeast glucose broth, upon aging, *mlt2* disruptants show a defect in polarized growth in the tip cells causing an enlarged tip and a swollen cell shape with two or four nuclei showing that nuclear division occurs without de novo cell wall synthesis. *Mlt2* mutants are hypersensitive to glucanase, indicating that the composition of the cell wall is affected by disruption of *mlt2*. Unlike the wild type, mutants do not produce aerial mycelium and do not melanize on PDA. The *mlt2* disruptants are reduced in virulence and cannot develop regular *septoria tritici* symptoms as they are unable to produce pycnidia. Cytological analysis of the disruptants *in planta* shows normal penetration of the germ tubes but subsequent branching and invasive growth are significantly hampered.

308. Distinct regulation and function of the thioredoxin proteins in Cryptococcus neoformans for oxidative and nitrosative stress or virulence. Tricia A. Missall[1] and Jennifer K. Lodge[1,2]. [1]Department of Biochemistry and Molecular Biology and [2]Department of Molecular Microbiology and Immunology, Saint Louis University School of Medicine

The thioredoxin system, consisting of thioredoxin, thioredoxin reductase, and NADPH, is known to protect cells against oxidative stress. This disulfide reducing system is present in *Cryptococcus neoformans* and consists of two small, dithiol thioredoxin proteins and one thioredoxin reductase. In this study, we describe the thioredoxin proteins, Trx1 and Trx2, and present their importance not only to stress resistance, but also to the virulence of *C. neoformans*. Using real time PCR, we show the induction of *TRX1* during oxidative stress and both thioredoxin genes during nitrosative stress. We show through deletion studies that the trx1delta mutant has a severe growth defect and is sensitive to oxidative and nitrosative stress, while the trx2delta mutant is only sensitive to nitric oxide stress. Using gene replacement studies, we demonstrate that the thioredoxin protein products are redundant in function and there is differential gene regulation which is important to nitrosative stress resistance. We have also identified two putative transcription factors, Atf1 and Yap4, which appear to differentially regulate the thioredoxin system under different conditions. Atf1 is necessary for oxidative stress induction and Yap4 is necessary for nitrosative stress induction of the thioredoxin genes in *C. neoformans*.

309. New functional genomic resource for *Candida albicans*. Aaron P. Mitchell, Qi Zhao, Frank J. Smith, and William C. Nierman. Microbiology Department, Columbia University, NYC, NY, and The Institute for Genomic Research, Rockville, MD

C. albicans, the most common human fungal pathogen, causes infections from oral and vaginal candidiasis to life-threatening invasive disease. We are using the recently published genomic sequence to identify virulence-related traits through insertional mutagenesis. We constructed a genomic *C. albicans* library, mutagenized the library with a Tn7-UAU1 transposon, and sequenced insertion sites to identify insertions within annotated ORFs. Among 32448 sequenced insertion sites, flanking sequences from 22199 clones have been mapped to 380 *Candida albicans* genomic contigs (Stanford release 19, with a total of 413 contigs) based on 85% length match, at least 85% identity, and the clone itself being at least 300 bp long. Within this group, we find insertions in 4170 (or 65.5%) of the 6362 unique ORFs defined by the NRCC annotation. A searchable insertion site database is available at http://www.tigr.org/tdb/e2k1/caa1/

We have examined a small group of insertion-bearing plasmids in detail to assess the feasibility of large-scale mutagenesis. We verified the insertion site sequences and determined endpoints of genomic DNA clones. We are currently transforming the pilot group of insertions into *C. albicans* to create heterozygotes, and will carry out UAU1-based selection to create homozygous insertion mutants.

We view this project as an important contribution to the *C. albicans* scientific community. In a broader sense, it serves as one model for cost-effective post-genomic functional analysis.

310. Matrix-Assisted Laser Desorption/Ionization Time-of Flight (MALDI-TOF)-based cloning of a MEKK like protein, CBCK1, from *Cryphonectria parasitica*. Ae-Young Mo, Eun-Sil Choi, Myoung-Ju Kim, Jie-Hye Kim, Seung-Moon Park, and Dae-Hyuk Kim. Division of Biological Sciences, Basic Science Research Institute, Chonbuk National University, Dukjindong 664-14, Jeonju,Chonbuk 561-756, Korea

On the foundation of a database of genome sequences and protein analyses, the ability to clone a gene based on a peptide analysis is becoming more feasible and effective for identifying a specific gene and its protein product of interest. As such, the current study conducted a protein analysis using 2-D PAGE followed by MALDI-TOF and ESI-MS to identify a highly expressed and phosphorylated protein spot. A highly phosphorylated protein spot with a molecular size of 44 kDa was selected and MALDI-TOF MS analysis was conducted. A homology search indicated that the protein appeared to be a fungal Bck1, which is a MEKK. Meanwhile, multiple alignments of fungal MEKK revealed a conserved amino acid sequence, from which degenerated primers were designed. Using the degenerate primers, we amplified and cloned a DNA fragment with a size of 369 bp. Sequence analysis of the clone revealed that it has the highest homology to the Bck1 like MEKK gene of *Podospora anserina*. Molecular characteristics of a gene encoding Bck1, *cbck*1, suggest that the *cbck*1 encodes the Bck1 homologue of *C. parasitica* and the functional analysis of the *cbck*1 is under performing.

311. Characterization of a *yap1*-related gene in *Ustilago maydis*. Lázaro Molina and Regine Kahmann. Max-Planck-Institut für terrestrische Mikrobiologie, 35043-Marburg, Germany

One of the most effective plant defense responses to pathogen attack is the production of reactive oxygen species (ROS). However, it is presently unknown how plant pathogenic fungi cope with these responses during a successful infection. Therefore, we became interested in studying ROS signalling in *Ustilago maydis*. In *Saccharomyces cerevisiae* has been extensively studied this signalling pathway and it has been shown that *YAP1* serves as central regulator for ROS signalling. *U. maydis* contains one *yap1* related gene. To analyse its role during pathogenic development, knockout mutants were generated in such way that the *yap1* ORF was substituted by the eGFP. In addition, *yap1:eGFP* fusions were constructed to localize the Yap1 protein during *U. maydis* development. The obtained mutants were significantly more sensitive to H_2O_2 than respective wild type strains and produced a dark pigment whose nature is currently unknown. This could indicate that under conditions where *yap1*-regulated genes are no longer expressed *U. maydis* is able to switch on the synthesis of other protectants. Compatible *yap1* knockout strains were able to mate, produced dikaryotic hyphae, invaded plants and induced disease symptoms. However, compared to the respective wild type strains virulence, of the *yap1* mutant strains was significantly attenuated. This was reflected by reduced number of tumors as well as reduced tumor size. These results indicate that *yap1* controls genes which allow *U. maydis* to cope efficiently with the plant environment. We are currently establishing which genes are regulated by *yap1* by microarray analysis. We will present this data and relate this to when and where Yap1 is active.

312. Mycotoxin production and fungal gene expression during infection of crowns and heads of wheat by *Fusarium graminearum*. A.M. Mudge, R. Dill-Macky*, Yan Hong*, I.Wilson and J.M. Manners. CSIRO Plant Industry, Queensland Bioscience Precinct, Brisbane 4072 and Canberra 2601, Australia; *Department of Plant Pathology, University of Minnesota, St. Paul, USA.

Fusarium graminearum (Fg) causes both Fusarium Head Blight (FHB) and Crown Rot (CR) of wheat in Australia. The mycotoxin deoxynivalenol (DON) has been reported to be important as a pathogenicity factor and grain contaminant in FHB but its role in CR is unknown. Following crown inoculation, hygromycin-resistant fungus was recovered from 100% of crowns, 85% of flag leaf nodes (FLN) and 55% of heads. Mycotoxin assays detected DON levels of 275 ppm in crowns, 14 ppm in FLN and 7 ppm in heads. Expression of Tri5 was strongly induced during crown infection. Results indicate that crown rot of wheat is associated with systemic fungal colonisation and DON production. To compare Fg gene expression in FHB and CR, a micro-array was printed that included 2300 clones from a subtracted FHB cDNA library and comparisons of gene expression were made between infected versus uninfected crowns and heads as well as to axenic cultured mycelium. The expression (normalised to fungal rRNA) of 48 selected infection-related fungal genes was then tested using qRT-PCR and 33 of these were validated as up-regulated during infection relative to their expression in cultured mycelium. Most were up-regulated in both diseases but 1 and 4 transcripts were only up-regulated in FHB and CR respectively. Most infection-related fungal genes were of unknown function.

313. Biological control of canegrubs, a proteomic approach. Helena Nevalainen[1,2], Nirupama Bhikballapur[1,2], Kathy Braithwaite[3], Stevens Brumbley[3], Peter Samson[4] and Junior Te'o[1,2]. [1]Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia. [2]Macquarie University Biotechnology Research Institute, Sydney, NSW, Australia. [3]BSES Limited, Indooroopilly, Qld, Australia. [4]BSES Limited, Mackay, Qld, Australia

The insect pathogenic fungus *Metarhizium anisopliae* is being developed as a biocontrol agent. However, attempts to discern the determinants of the infection process and produce a rational plan for strain improvement have been thwarted by the complexity of host-target related signals. Therefore, broad strategies are needed to pursue the functional genome of *M. anisopliae*. This study focuses on the identification of key proteins during infection of sugarcane whitegrub, the most serious insect pests of sugarcane in Australia by *M. anisopliae* var. *anisopliae*.
The experimental setup is designed to mimic the infection process in the field. Proteins were extracted from samples representing healthy and infected canegrubs, healthy and infected insect cuticles and fungal mycelia. The extracted proteins were separated by 1DE and 2DE and samples assessed by comparative analysis in order to identify the key proteins involved in pathogenicity. As an example, the protein profile from infected insect cuticles displayed a notable absence of higher molecular weight proteins present in non-infected cuticles. Further comparative analysis of the protein profiles will be presented with some identifications of unique "signatory" proteins for each condition.

314. Alpha cells predominate in the mammalian central nervous system during *Cryptococcus neoformans* mixed mating type infections. Kirsten Nielsen and Joseph Heitman. Duke University Medical Center, Howard Hughes Medical Institute, Durham, NC

Cryptococcus neoformans is an opportunistic human pathogen that infects the central nervous system to cause meningitis that is uniformly fatal if untreated. This basidiomycete has evolved over the past 40 million years into three distinct varieties or sibling species (*grubii*, *gattii*, and *neoformans*). The three *Cryptococcus* varieties have different disease epidemiologies with var. *grubii* producing 95% of human disease. *C. neoformans* is a heterothallic fungus with two mating types - **a** and alpha. Interestingly, the vast majority of clinical isolates are alpha mating type with **a** strains accounting for only a limited proportion of isolates. It is unclear why alpha strains predominate in clinical samples because our studies with var. *grubii* congenic strains showed that **a** and alpha strains have equivalent virulence in cellular (macrophage), heterologous host (amoeba, nematode), and mammalian (mouse, rabbit) models of cryptococcosis. Humans are thought to be exposed to *C. neoformans* via inhalation of small yeast cells or spores - resulting in an initial pulmonary infection that hematogenously disseminates to the central nervous system in immunocompromised individuals. We have shown that **a** and alpha strains cause disease in a similar fashion when infected individually. In contrast, coinfection studies with the **a** and alpha congenic strains revealed equivalent levels of **a** and alpha cells in the lungs but a significantly higher proportion of alpha cells infecting the brain. Thus, the alpha strain out-competes the **a** strain during a mixed infection of the central nervous system. These results provide an explanation for why alpha strains are the predominant clinical isolates from human cerebrospinal fluid, lay the foundation for detailed studies on the interaction of **a** and alpha strains *in vivo*, and provide a mechanism to define virulence characteristics important for central nervous system infection.

315. Linkage between Mitochondrial Hypovirulence and Viral Hypovirulence in the Chestnut Blight Fungus, *Cryphonectria parasitica*, revealed by cDNA Microarray Analysis. Donald L. Nuss and Todd D. Allen, Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742.

The phenomenon of transmissible hypovirulence (virulence attenuation) associated with biological control of natural populations of the chestnut blight fungus *Cryphonectria parasitica* can be experimentally reproduced by infection with hypovirus cDNA clones (viral hypovirulence) or by mutation of mitochondrial (mt) DNA in the absence of virus infection (mitochondrial hypovirulence). We now report the use of an established *C. parasitica* cDNA microarray to monitor nuclear transcriptional responses to a mtDNA mutation of *C. parasitica* strain EP155, designated EP155/mit2, previously shown to induce elevated alternative oxidase activity and hypovirulence [MonterioVitorello, C. B., Bell, J. A., Fulbright, D. W. & Bertrand, H.A. (1995) Proc. Natl. Acad. Sci. 92,5935-5939]. Approximately 10% of the 2,200 genes represented on the microarray exhibited altered transcript accumulation as a result of the mit2 mtDNA mutation. While genes involved in mitochondrial function were clearly represented in the EP155/mit2 responsive gene list, direct parallels to the well-characterized *Saccharomyces cerevisiae* retrograde response to mitochondrial dysfunction were not observed. Remarkably, 47% of the genes that were differentially expressed following infection of strain EP155 by the prototypic hypovirus CHV1-EP713 were similarly changed in transcript accumulation in virus-free EP155/mit2. These results establish a linkage between viral and mitochondrial hypovirulence and raise questions regarding the relationship between hypovirus infection and mitochondrial dysfunction. The results are discussed in terms of mitochondria-to-nuclear communications in the context of hypovirus infection and fungal pathogenesis.

316. Metabolic requirements for pathogenicity. RP Oliver, R Lowe, PS Solomon, R Trengove, R Lee, O Waters, J Rechberger. Australian Centre for Necrotrophic Fungal Pathogens

The ability of a fungal pathogen to acquire nutrients from its plant host is a key factor in the success of its lifecycle. An understanding of these processes may help us design new strategies to combat pathogens. Our current knowledge of this subject is limited and in many cases established dogma has remained unchallenged.
We study the wheat pathogen *Stagonospora nodorum*, by combining a study of specific gene knockouts with metabolomic analyses. GC-MS analyses detect 100-200 individual compounds on both in vitro and in planta samples.
Knockouts of malate synthase (MS), glyoxalase, a peptide transporter and mannitol 1-phosphate dehydrogenase (MPD)have been studied. Deletion of the peptide transporter had no obvious phenotypic consequences indicating that peptides do not constitute a significant nutrient source in planta. Likewise, deletion of the glyoxalase gave no pathophenotype but did result in strains that were less tolerant of methylglyoxal, the presumed substrate. Deletion of the MPD gene gave strains that were still capable of producing lesions but had reduced ability to sporulate in planta. This indicate that mannitol metabolism may have a role in ameliorating the stress produced by senescing leaves and thereby promoting sporulation. MS mutants were completely non-pathogenic as they were incapable of germinating on the leaf (although glucose could restore this), indicating that gluconeogenesis is critical in the early phases of infection.
This work is supported by the Australian Grains Research and Development Corporation

317. Effects of a Plant-Pathogen Interaction on the Fungal Endophyte Community. Jean J. Pan[1] and Georgiana May[1,2]. [1]Department of Ecology, Evolution, & Behavior, [2]Department of Plant Biology, University of Minnesota, Saint Paul, MN 55108, USA

Humans have been modifying plant traits directly through breeding or indirectly through changes in the environment. Changes in plant traits affect not only plant fitness but can also affect microbes that interact with the plants. We investigated the effects of host plant variation for pathogen resistance and the effects of pathogen infection on the fungal endophyte community in the *Zea mays-Ustilago maydis* (corn-corn smut) system. We used a culture-independent approach to assess the fungal endophyte community on three recombinant inbred lines (RILs) of corn resistant to corn smut and three RILs susceptible to corn smut. We found that neither host trait variation or smut infection had consistent effects on endophyte species richness and species diversity. Instead, effects of smut infection seemed to vary with RIL, with three RILs having greater endophyte species richness and species diversity when infected by corn smut. Data on the community composition of fungal endophytes in relation to host trait variation, smut infection, and corn line will also be examined.

318. A proteomics-based approach for identification of the *ToxD* gene. Iovanna Pandelova and Lynda M. Ciuffetti. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA.

Several host-selective toxins (HSTs) are produced by the wheat pathogen *Pyrenophora tritici-repentis*, including Ptr ToxA, Ptr ToxB and Ptr ToxC. The race structure of *P. tritici-repentis* is defined by the production of one or a combination of HSTs by a particular isolate and its interaction with a specific set of wheat differentials. Ptr ToxD, a recently isolated proteinaceous toxin, exhibits the same specificity as Ptr ToxA on the currently designated set of wheat differentials. The race 9 isolate, which produces Ptr ToxD and not Ptr ToxA, was identified only when polymerase chain reaction (PCR) and Southern blot analysis revealed the lack of the *ToxA* gene. As isolate-by-isolate protein purification promises to be tedious and time consuming, screening isolates for the presence of the *ToxD* gene first followed by confirmation of Ptr ToxD production provides a more efficient approach. In order to identify and clone the *ToxD* gene, we used a proteomics-based approach. Ptr ToxD activity was purified to a single 10 kDa protein band as determined by SDS-PAGE and confirmed by MALDI mass spectrometry. Purified protein was digested with trypsin. The resulting peptides were separated with a Waters CapLC system and subsequently analyzed with an electrospray ionization (ESI) quadrupole time-of-flight (Q-TOF) Global system mass spectrometer. Degenerate primers were designed based on peptide sequences and used in reverse-transcriptase PCR to amplify a partial cDNA clone from a race 9 isolate. This amplification product will be used to isolate and clone the gene.

319. The signal peptidase of *Colletotrichum graminicola*, and its role in pathogenicity to maize stalks and leaves. E. Park and L. Vaillancourt. University of Kentucky.

The fungus *Colletotrichum graminicola* causes anthracnose disease on maize. The anthracnose syndrome includes a leaf blight phase, as well as a more damaging and less understood stalk rot. A *C. graminicola* mutant was identified in our laboratory that colonized normally as a biotroph in leaves, but failed to make the transition to necrotrophic growth. The mutant was also significantly reduced in its ability to colonize and rot living stalks. The predicted mutant protein, CPR1, has 37% similarity to pSPC3, one of four subunits of the signal peptidase of yeast. Our current model is that the mutant is unable to secrete necessary proteins to sustain necrotrophic growth. Our goal was to test the hypothesis that CPR1 is a component of the *C. graminicola* signal peptidase. The *Cpr1* cDNA was cloned and transformed into two different yeast *spc3* mutants. However, CPR1 did not complement either mutant yeast strain, perhaps because CPR1 is too divergent from pSPC3. To obtain indirect evidence for the function of CPR1, a gene encoding a putative pSEC11 homologue of C. graminicola (*Cgs11*) was cloned and characterized. pSEC11 is one of four subunits of the signal peptidase in yeast, and has been shown to physically interact with pSPC3. The predicted protein encoded by the *Cgs11* cDNA was 67 % similar to pSEC11. The *Cgs11* cDNA complemented two different yeast *sec11* mutants. Co-immunoprecipitation and co-immunolocalization experiments are currently in progress to test for an interaction between CGS11 and CPR1.

320. Mechanisms of differential spore germination and growth of *Fusarium oxysporum* in the root of *Arabidopsis thaliana*. Sook-Young Park[1], Yin-Won Lee [2], Jim Tumlinson [3], Jurgen Engelberth [3], Seogchan Kang[1]. [1] Department of Plant Pathology and [3] Department of Entomology. The Pennsylvania State University, University Park, PA 16802. [2] School of Agricultural Biotechnology, Seoul National University, 151-742, Korea.

The soil-borne fungus *Fusarium oxysporum* causes vascular wilt in a wide variety of plant species, including *Arabidopsis thaliana*. We studied the germination of *Fusarium oxysporum* spores in the rhizosphere of various *Arabidopsis* ecotypes and the subsequent colonization of the root system using a fluorescently labeled strain. Two Arabidopsis ecotypes, Cape Verde (CV) and Greenville (GRE), differentially responded to O-685, a cabbage isolate. Seedlings of GRE inoculated with O-685 showed no external signs of disease, whereas CV seedlings were severely stunted and showed typical Fusarium wilt symptoms. Spores poorly germinated in the rhizosphere of GRE, whereas the germination rate in the rhizosphere of CV was high. Plant root exudates initiate and manipulate interactions with soil microbes, including *F. oxysporum*, through the secretion of various primary and secondary metabolites. We hypothesize that the root exudates from these ecotypes are significantly different in their composition. Metabolite analysis to identify chemical basis of differential spore germination is in progress.

321. Proteins involved in the apple scab disease interaction. Plummer KM[1], Fitzgerald AM[1,2], van Kan, JAL[3], Greenwood DR[2], Cui, W[2], Templeton MD[2]. [1]University of Auckland, PB 92019, NZ. [2]HortResearch, Mt Albert Research Centre, PB 92169, NZ. [3]Plant Sciences, Wageningen University, Netherlands.

The biotrophic fungus *Venturia inaequalis* causes apple scab disease. Resistance to *V. inaequalis* conforms to a gene-for-gene model and is due to the presence of major resistance genes in the Malus host and avirulence genes in the fungus. It is hypothesised that secreted fungal proteins are involved in the maintenance and specificity of scab disease. We are using genomics and proteomics approaches to identify avirulence and pathogenicity proteins secreted by the fungus, and plant proteins whose expression is influenced by the pathogen. Proteins isolated from infected apple leaf tissues are being analysed using ESI-MS/MS. Peptide fragment data and sequence information is then being screened against an EST (expressed sequence tag) database from *V. inaequalis*-infected leaf material, and from *in vitro* grown *V. inaequalis*. Genes and proteins of interest including: proteins identified from infected leaf tissue; proteins that elicit specific host resistance responses; genes differentially expressed on cellophane and *in planta* compared with growth on nutrient media; and ESTs with secretory peptides, are being screened in functional assays and using real-time PCR. We have developed gene silencing in *V. inaequalis* to facilitate the functional characterisation of genes of interest.

322. Tissue acidification and ROS accumulation during pathogenicity of *Penicillium expansum*. Yoav Hadas[1], Israel Goldberg[2], Ophry Pines[2] and Dov Prusky[1]. [1]Department of Postharvest Science of Fresh Produce in the Institute of Agricultural Products, ARO, Volcani center, Bet Dagan 50250 and [2]Department of Molecular Biology, Hebrew University, Medical School, Jerusalem 91120, Israel.

The phytopathogenic fungus *Penicillium expansum* acidifies the host tissue and consequently enhanced *pepg*1 expression, a gene encoding for polygalacturonase, involved in host maceration and fungal attack of *Penicillium expansum*. The acidification is achieved by secretion of organic acids mainly gluconic acid. *P. expansum* isolates with increased pathogenicity, accumulated higher amounts of gluconic acid and reduced the apple tissue pH to lower values than isolates with reduced pathogenicity. Glucose oxidase activity, involved in gluconic acid production, was detected in *P. expansum* decayed tissue but not in the healthy tissue of the same fruit. Glucose oxidase activity and accumulation of gluconic acid were strongly affected by environmental pH conditions. Reactive oxygen species resulting from glucose oxidase activity were easily detected in the decayed apple tissue and specifically in the hyphae. It is suggested that acidification of host tissue by organic acids is an important factor involved in host maceration and fungal attack of *P. expansum*.

323. Withdrawn

324. Regulation of gene expression on *Colletotrichum gloeosporioides* by alkalinization. Itay Miyara[1], Amir Sherman[2] and Dov Prusky[1]. [1]Department of Postharvest Science of Fresh Produce and [2]Department of Genomics, Agricultural Research Organziation, Volcani Center, Bet Dagan 50250

C. gloeosporioides is an important pathogen of tropical and subtropical fruits. During the colonization the pathogen alkalinize the host tissue by secreting significant amount of ammonia. The alkalinization of the host tissue enhanced *pel*B expression, a gene encoding for pectate lyase that affect colonization of fruits and possible other genes that were not identified. As a first stage for identification of the genes expressed during the ammonification process and their contribution to fungal pathogenicity we prepared cDNA libraries of *C. gloeosporioides* grown at inducing pH *in vitro* and in semi *in vivo* on avocado fruits. The cDNA libraries were printed on nylon membranes in a macroarray format. The macroarrays were used to determine the differential expression of genes under inducing and non inducing conditions of colonization. Results from the macroarray experiments will be shown. We intend to use these results for gene knockouts and identification of new non-pathogenic mutants

325 Isolation of a Novel DNA Sequence Required for Pathogenicity of *Colletotrichum* Species. Regina S. Redman and Rusty Rodriguez. University of Washington, Biology, Seattle, WA. Montana State University, Bozeman MT. U.S. Geological Survey, Seattle, WA.

We have isolated a unique DNA sequence (designated *fsl*-1, fungal symbiotic lifestyle) from ,*Colletotrichum magna*, a cucurbit pathogen, that is required for pathogenesis and is conserved in the genus *Colletotrichum*. The *fsl*-1 sequence is not currently represented in public sequence databases and was used to generate a targeted gene disruption vector (pGD13). Transformation studies involving six *Colletotrichum* species resulted in the conversion of virulent pathogens to non-pathogenic endophytic mutualists. The lifestyle altered transformants asymptomatically colonized plants and conferred disease resistance against the virulent wildtype isolates. Southern blot analysis revealed that pGD13 disrupted *fsl*-1 by homologous integration. Expression studies indicated that *fsl*-1 is expressed in culture in non-pathogenic transformants but not in virulent wildtype isolates. Collectively, these results indicate that *fsl*-1 is required for the expression of pathogenic lifestyles in *Colletotrichum* species. Studies are currently underway to determine the expression of *fsl*-1 *inplanta* and determine the ubiquity of *fsl*-1 in other fungal genera.

326. Genes Expressed During Early Infection of *Arabidopsis thaliana* by *Ustilago maydis*. Cristina G. Reynaga-Peña[1] , Lucila Méndez-Morán[1,2] , Barbara Jablonska[3] , Patricia S. Springer[3] and José Ruiz-Herrera[1]. [1]Centro de Investigación y de Estudios Avanzados del I.P.N., Unidad Irapuato, MÉXICO, [2] Depto. de Ecología CUCBA, Universidad de Guadalajara, MEXICO and [3] University of California, Riverside, Riverside, CA, U.S.A.

We used two approaches to identify *A. thaliana* up-regulated genes during the early stages of infection by *U. maydis*: 1) screen of enhancer/genetrap lines from *A. thaliana*, and 2) preparation of an expression subtractive library. Through the screen of 1219 Arabidopsis lines from the UCR trap collection, we identified lines that showed an alteration in GUS reporter gene expression in response to fungal infection. TAIL-PCR was used to identify the genes tagged in these lines. We found insertions into genes encoding known and unknown proteins: a putative sulfate transporter, a predicted transcription factor, chloroplast-related genes, and others. A comparison of these results with screens made by colleagues on the same UCR lines allowed us to dissect general biotic from abiotic stress responses, and those that were specific for fungal infection. In a parallel approach, a subtractive library was obtained from RNA expressed after 24 or 96 h post-infection, and so far we have identified two up-regulated genes, one encoding the CP12 protein from chloroplast, and a serine protease inhibitor. Additionally, we searched on available databases to find if some of the *A. thaliana* genes up-regulated during *U. maydis* infection were also regulated during infection by other plant pathogens, including bacteria and fungi. This work is important for the identification of *A. thaliana* genes that may play an important role either in plant defense against *U. maydis* infection, or involved in the changes in plant growth that occur during infection. We are currently investigating these phenomena using different approaches.

327. Triacylglycerol Lipase 1 Gene of *Magnaporthe grisea* is Essential for Appressoria Turgor and Pathogenic Growth in Host Plant Cells. Hee-Sool Rho[1], Soonok Kim[1], ChangHyun Khang[2], Seochan Kang[2], and Yong-Hwan Lee[1]. [1]School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea. [2]Department of Plant Pathology, The Pennsylvania University, University Park, PA 16802, USA.

Magnaporthe grisea elaborates a specialized infection structure, called an appressorium, which is used to rupture the plant cuticle by mechanical force. Appressoria generate turgor pressure by accumulating high concentration of glycerol. Triacylglycerol lipase activity increased during appressoria maturation. To investigate the molecular genetic roles of triacylglycerol lipase on appressorium formation and turgor generation, we characterized the function of triacylglycerol lipase 1 gene (*LIP1*) by a gene disruption strategy. The *lip1* mutants showed impaired properties on conidial germination and adhesion. Appressorium formation of *lip1* mutants was retarded and reduced, which seemed to be associated with turgor pressure generation. *lip1* mutants also showed reduced virulence on host plant. Defects on conidial germination and appressorium formation of *lip1* mutants were restored by exogenous addition of diacylglycerol. These suggest that *LIP1* plays important roles on infection-related morphogenesis and turgor generation in *M. grisea*. These results will provide further understanding of the relationship between lipid metabolism and turgor generation for fungal pathogenesis

328. Identification of cyst surface proteins from the fish pathogen *Saprolegnia parasitica*. Emma Robertson and Pieter van West. The Aberdeen Oomycete Group, College of Life Sciences and Medicine, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, Scotland, UK.

Fish pathogens, such as the oomycete *Saprolegnia parasitica*, cause devastating damage and loss of profit in the fish farming industry worldwide. These pathogens cause Saprolegniosis, a disease where filamentous mycelium grows into the fins and body of freshwater fish. The disease results in slow and sluggish movement of infected fish. Severe infection may result in death of the host. Very little is known of the molecular biology of *S. parasitica*, and pathogenicity of the oomycete is undetermined. It has been proposed that spines present on the surface of secondary zoospores hook onto the scales of the fish, and aid in the initial host-pathogen interaction. This would then permit an opportunity for further invasion and colonisation.
Here we present a proteomic approach to identify hook proteins and other surface proteins that may play a role in pathogenicity. Furthermore, we are optimising an RNA interference (RNAi) protocol for *S. parasitica*. Using RNAi, it should be possible to perform functional analysis of genes in *S. parasitica*. By having a better understanding of the proteins involved in the pathogenicity of *S. parasitica*, it may be possible to identify potential drug targets and develop a new route of controlling this devastating, and economically important disease.

329. Construction of a physical and fingerprint fragment restriction map of a conditionally dispensable chromosome in *Nectria haematococca*. Marianela Rodriguez, Dai Tsuchiya, and Hans D. VanEtten. Division of Plant Pathology and Microbiology, Department of Plant Sciences, University of Arizona.

Nectria haematococca has a wide range of habitats, although individual isolates have a limited habitat range. Part of this habitat diversity is due to the presence of supernumerary chromosomes that contain unique habitat defining genes. Since these chromosomes are not needed for axenic growth but are important for habitat specificity, they have been called "Conditionally Dispensable" (CD) chromosomes. One of these CD chromosomes in *N. haematococca* contains a cluster of genes for pea pathogenicity called the *PEP* cluster. It has been suggested that the genes in the *PEP* cluster, as well as the CD chromosome, are of foreign origin, due to differences in GC content and codon usage when compared to the genes from other chromosomes of *N. haematococca*.
In the current study, we present a partial physical map of the 1.6 Mb CD chromosome of *N. haematococca*. The fingerprint data for the map were obtained by restriction enzyme digestion of cosmid clones from a CD-specific library. Contigs were generated by analyzing overlapping clones using the Arizona Genomics Institute's FPC software. Contig arrangements are being identified by fiber fluorescence *in situ* hybridizations. This CD chromosome contig map will be used for studies of syntony and recombination events, which could lead to a better understanding of the origins of CD chromosomal DNA.

330. *Claviceps purpurea* – Signalling in Directed Growth. Yvonne Rolke, Jan Scheffer and Paul Tudzynski. Institute of Botany, Westfaelische Wilhelms-Universitaet Muenster, Schlossgarten 3, 48149 Muenster, Germany

An interesting model to study directed growth of fungal hyphae in a pathogen host interaction is represended by the biothrophic ascomycete *Claviceps purpurea* on rye.
Two approaches will reveal new aspects for the understanding of directed growth mechanisms in this system.
In a forward genetics approach the creation of an insertional mutant library based on the Agrobacterium-mediated T-DNA-transfer is in progress. Mutants with impaired growth can be identified easily in an in vitro system for cultivation and infection of rye ovaries.
In a reverse genetics approach we are interested in the analysis of signal chain components. It was shown by J. Scheffer that *cpcdc42*, a small GTPase, is a prerequisite for directed growth of *C. purpurea* in the infection process. In yeast Cdc42 activates among other proteins PAK kinases. PAK kinases are known to influence hyphal growth in several fungi. Deletion of the PAK kinase gene *cpcla4* revealed a striking phenotype.*Cpcla4* deletion mutants grow in a corral like shape with shortened, blistered cells which contain big vacouls. A closer look to Cpcdc42 and Cpcla4 downstram acting elements in *C. purpurea*, via a macroarray approach which contains nearly 10.000 sequenced and spotted genes is underway. The analysis will contribute to the understanding of elements which are involved in the unique system of directed growth and polarisation during the infection process of *C. purpurea*.

331. Multidrug resistance in *Mycosphaerella graminicola* involves the novel major facilitator superfamily (MFS) transporter gene *MgMfs1*. Ramin Roohparvar[1], Lute-Harm Zwiers[1], Gert H.J. Kema[2], and Maarten A. De Waard[1]. [1]Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands. [2]Businessunit Biointeractions and Plant Health, Plant Research International, Wageningen, The Netherlands

Screening of EST libraries from the wheat pathogen *Mycosphaerella graminicola* led to the identification of *MgMfs1*, a full length Major Facilitator Superfamily (MFS) gene with high homology to putative toxin transporters involved in virulence. Complementation of a *Saccharomyces cerevisiae* strain carrying multiple non-functional drug transporter genes, with *MgMfs1* resulted in an impressive decrease in sensitivity of *S. cerevisiae* to a broad range of synthetic and natural toxic compounds. *MgMfs1* disruptants of *M. graminicola* generated by *Agrobacterium tumefaciens*-mediated transformation showed an increased sensitivity to strobilurin fungicides and to the mycotoxin cercosporin, whereas no reduction in virulence on wheat seedlings could be observed. The results indicate that the encoded protein MgMfs1 is a true multidrug transporter that can function as a determinant of the pathogen in sensitivity and resistance to fungicides, and that might play a role in secretion of mycotoxins.

332. Lost in the middle of nowhere: the avirulence gene *AvrLm1* of *Leptosphaeria maculans*. Lilian Gout[1], Simon Ross[1], Marie-Line Kuhn[1], Françoise Blaise[1], Laurence Cattolico[2], Thierry Rouxel[1], Marie-Hélène Balesdent[1]. [1] INRA, PMDV, F-78026 Versailles Cedex, France; [2]Genoscope, Centre National de Séquençage, 91057 Evry cedex, France.

The stem canker fungus, *Leptosphaeria maculans*, develops gene-for-gene interactions with its Brassica hosts, and avirulence genes appear to be genetically clustered in the fungal genome. Map-based cloning of *AvrLm1* was initiated and currently represents one of the longest walks performed in fungi. It encountered all possible troubles, including under representation in libraries and hundreds-of-kb stretches of repeats. *AvrLm1* was located within one 630 kb *Not*I restriction fragment, fully covered by a BAC contig comprising 11 clones. The region was bordered by ORF-rich clusters, and encompassed one 35-kb ORF-rich region. The rest of the region consisted of large stretches of A+T-rich composite and degenerated repeats, and the candidate *AvrLm1* was a solo gene surrounded by 175 kb of repeats on its 5' side and 90 kb of repeats on its 3' side. Functional complementation of a virulent isolate with the ORF and its putative promoter region fully restored the avirulent phenotype on a range of *Rlm1* oilseed rape genotypes. Analysis of natural populations historically submitted to various levels of *Rlm1* selection pressure indicated one highly predominant mechanism for loss of avirulence, i.e. deletion of an average 180 kb of the region encompassing *AvrLm1*.

333. Microarray transcription profiling provides insights into metabolic regulation and stress adaptation during infection of wheat by the Septoria leaf blotch pathogen, *Mycosphaerella graminicola*. Jason Rudd*, John Keon, John Antoniw, Wendy Skinner, John Hargreaves and Kim Hammond-Kosack*. Wheat Pathogenesis Programme, Plant-Pathogen Interactions Division, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK

Septoria leaf blotch disease is amongst the most important fungal diseases of cultivated wheat and is caused by the ascomycete fungus *Septoria tritici* (anamorph *Mycosphaerella gramincola*). Studies on host-pathogen interactions at the transcriptome level have predominantly been restricted to investigations of host responses. Here we present one of few examples of a global transcriptome analysis of a fungal pathogen performed during a natural encounter with its susceptible host. A *Mycosphaerella graminicola* EST-based microarray has been developed and used to compare the nutrition and development of the fungus *in vitro* under nutrient rich and nutrient limiting conditions and *in vivo* at a late stage of host infection. The data obtained have provided insights into; (1) the direction and utilisation of primary carbon flux in the three environments; (2) high and low nutrient transcriptional activation and / or repression events indicative of the nutrient sources available *in planta*; (3) elevated stress adaptation / tolerance in the host environment; (4) differential expression of fungal virulence / pathogenicity factor homologues. We are also able to conclude that with respect to primary metabolism and nutrient availability, late stage fungal growth *in planta* more closely resembles that in a nutrient rich, as opposed to a nutrient limiting, environment.

334. Novel genes specifically expressed at early infection stage of rice blast fungus. K. Saitoh[1], F. Ishii[1], M. Kanamori[1], T. Yamashita[1], T. Arie[1], T. Kamakura[2], and T. Teraoka[1]. [1]Tokyo Univ. of Agric. & Techn., Fuchu, Tokyo, Japan. [2]Tokyo Univ. of Science, Noda, Chiba, Japan.

The conidial germ tube of the rice blast fungus, *Magnaporthe grisea*, differentiates an appressorium, essentially required for penetration into the host plant. In our differential cDNA library of *M. grisea* strongly expressed during appressorium formation, unique and distinct clones were contained. From the library we have found and reported the novel *CBP1* gene involved in sensing physically the surface on which the conidia attached. Additionally we found novel genes, *B19*, *B48* and *B59*, specifically expressed in appressorium formation. Bioinformatic analyses suggested that these genes could encode sucrase/ferredoxin-, dual specificity phosphatase- and glycoside hydrolase (GH61)-like protein, respectively. These genes were disrupted by homologous recombination to elucidate the functions of the genes. The null mutants tested normally grew on YG medium, and sporulated on oatmeal medium. But the *B19* and *B48* mutants significantly decreased the ability to form the appressorium on polycarbonate plates, although the conidia normally germinated. Chemical inducers to trigger the appressorium formation, 1,16-hexadecandiol as a cutin monomer, cAMP and IBMX (an inhibitor of degradation of cAMP), restored the ability of the appressorium formation on the plate. Both *B19* and *B48* mutants decreased the ability of penetration and infectious growth in host cells, although they did not completely lose the ability to develop lesions in spray and punch-wounded inoculation. These results suggest that the genes set required for appressorium formation, penetration, and initial infectious growth by establishment of colonization in host cells may be quite different from the genes set after the establishment to develop the visible lesions, and that *B19* and *B48* genes may be involved in the earlier infection steps. Function(s) of *B59* was also elucidated by the similar ways.

335. The Gpmk1 MAP kinase regulates the secreted lipase FGL1, a novel virulence factor of *Fusarium graminearum*. Attila Gacser, Christian A. Voigt, Nicole J. Jenczmionka, Siegfried Salomon, and Wilhelm Schaefer. Department of Molecular Phytopathology and Genetics, University of Hamburg, Germany.

Mitogen activated protein (MAP) kinases play important roles during different developmental processes including pathogenic stages of many filamentous fungi. It has been reported that Gpmk1 MAP kinase disruption mutants of *Fusarium graminearum* are apathogenic and cannot infect wheat spikes. At this time it is not possible to explain the complete apathogenicity of the MAP kinase deletion mutants, because the Gpmk1 MAP kinase affects several processes in the cells.
An effective fungal pathogen must overcome physical and chemical barriers made up by the host to block infection. The mode of penetration and invasion of *F. graminearum* is still not fully elucidated. However, the formation of appressoria has been excluded, as such structures were never found. Instead, the fungus probably enters the host through natural openings, such as the glume stomata, or penetrates the epidermal cell walls directly with short infection hyphae. *F. graminearum* secretes cell wall degrading enzymes during colonization of its host. Jenczmionka and Schaefer (Curr. Genet., 2004) could show that the regulation of various cell wall degrading enzymes is mediated by the MAP kinase pathway. Gpmk1 MAP kinase disruption mutants of *F. graminearum* show *in vitro* a reduced lipolytic activity in comparison to the wild type strain. Here we show the regulation of the secreted lipase FGL1 in dependence of MAP kinase Gpmk1.

336. Visualizing the impact of trichothecenes on the development of *Fusarium* Head Blight. Frank J. Maier, Hadeler, B., and W. Schaefer. Department of Molecular Phytopathology and Genetics, Biocenter Klein Flottbek, University of Hamburg, Germany.

Head blight (FHB) caused by the ascomycetic fungus *Fusarium graminearum* (*Gibberella zeae*) is one of the most destructive diseases of cereals. It causes yield reductions and contaminates grain with mycotoxins, which constitutes a potential risk for human and animal nutrition. One important class of mycotoxins produced by several *Fusarium* species are the trichothecene derivatives (e.g. nivalenol, deoxynivalenol). Trichothecenes accumulate in *Fusarium*-infested food and non-specifically affect most eukaryotes.
To depict the importance of the trichothecenes on spreading and development of FHB we expressed constitutively the gene for the green fluorescent protein (gfp) in *F. graminearum* wild type and tri5 disruption mutants, the latter carry the gfp cassette in the disrupted tri5 gene. To evaluate the induction of the trichothecenes in the host plants, the gfp gene was fused to the tri5 promoter and integrated into the genome by homologous recombination. The resulting mutants exhibit a gfp gene driven by the endogenous tri5 promoter and a fully functional tri5 gene. Monitoring gfp expression, we evalute the infection of wheat spikes by wild type and trichothecene deficient mutants as well as the induction of the trichothecene pathway.

337. Surprising variation to the general scheme: The mating type genes of *Sporisorium reilianum*. Jan Schirawski, Bernadette Heinze, Martin Wagenknecht and Regine Kahmann. Max-Planck-Institute for terrestrial Microbiology, Marburg, Germany.

Sporisorium reilianum causes head smut of maize and sorghum. Infection takes place at seedling stage with disease symptoms appearing only in the inflorescence. With respect to the infection process *S. reilianum* is very similar to the related species *Ustilago hordei* and *U. avenae*. However, genetically, *S. reilianum* is more closely related to *U. maydis* that can induce disease symptoms on all green parts of the maize host. In *U. maydis*, mating and pathogenesis are regulated by a pheromone/receptor system and a pair of homeodomain proteins, which are encoded on two unlinked mating type loci, *a* and *b*. Mating and sexual development require that sporidia differ at both mating type loci.
To elucidate the differences in infection mode of the closely related smut fungi *S. reilianum* and *U. maydis*, we have investigated the mating type loci of *S. reilianum*. We isolated haploid sporidia from spore samples collected on different continents. In contrast to published reports, which state that *S. reilianum* has four mating types, we found five different *b* alleles and three different *a* alleles. We have sequenced all eight loci. Both the *a* and *b* alleles show high synteny to the respective loci of *U. maydis*. However, the *a* alleles contain one additional pheromone precursor gene each. With respect to specificity only three different functional mating pheromones exist, with two of the three being present on any one locus. This is the first report of a smut fungus having three different *a* alleles, i.e. three different pheromone/receptor systems.

338. The role of 'Necrosis and Ethylene inducing Proteins' (NEPs) of *Botrytis cinerea* in plant cell death and pathogenicity. Alexander Schouten, Yaite Cuesta Arenas, Peter van Baarlen & Jan van Kan. Wageningen University, Laboratory of Phytopatholgy, Wageningen, The Netherlands

The plant pathogenic fungus *Botrytis cinerea* is considered a necrotroph, since it kills the host tissue prior to colonization. Little information about the mechanism of cell killing is available but it was proposed to involve (possibly concerted) action of diffusible toxins, oxalic acid, cell wall degrading enzymes and the production of active oxygen species (AOS).
We characterized two genes in *B. cinereaI*, *Bcnep1* and *Bcnep2*, which are both homologs of the 'necrosis and ethylene inducing protein' gene (nep1) initially described in *Fusarium oxysporum,* f.sp. *erythroxyli*. Currently, approximately 25 NEP homologs have been characterized in various bacteria, oomycetes and fungi. Generally, NEPs are phytotoxic for dicotyledons. In hemibiotrophic plant pathogens, the *nep* genes are expressed during the onset of the necrotrophic phase. However, it is currently still not clear whether NEPs do play a key role in pathogenicity.
The *Bcnep1* and *Bcnep2* genes are differentially expressed during the infection of tomato, suggesting specific roles for each protein during pathogenesis. When synthesized in heterologous expression systems, both BcNEP1 and BcNEP2 have phytotoxic activity. Prior to cell death, tobacco leaves respond by an increase in ethylene production and the accumulation of AOS. Currently, *Bcnep* disruption mutants of *B. cinerea* are generated. The mechanism of plant cell death and the role of these proteins in pathogenesis is discussed.

339. Physiological and molecular responses of *Alternaria brassicicola* to exposure to host plant antimicrobial compounds. Adnane Sellam, Pascal Poupard, Philippe Simoneau. UMR PaVé N°77, Faculté des Sciences, 2 Bd Lavoisier, 49045 Angers cedex, France

Alternaria brassicicola is a necrotrophic seed-borne pathogen responsible for the black spot disease of crucifers. During the colonization of the host tissues, this fungus is exposed to several antimicrobial plant compounds such as phytoanticipins and phytoalexins. In order to better understand how the fungus deals with such toxic environment, we first analyzed in vitro the effects of two glucosinolate-derived isothiocyanates (ITC) and of camalexine (CAM) on different growth parameters of *Alternaria brassicicola* . Briefly, allyl-ITC and to a lesser extent benzyl-ITC strongly inhibited *Alternaria brassicicola* developement. Concerning CAM, inhibitory effects on spore germination and germ-tube elongation were observed at concentrations significantly lower than those measured in planta. At higher concentrations, effects on radial growth and sporulation were also recorded. In a second step, we analyzed the expression of fungal target genes after exposure to ITC or CAM at sub-inhibitory concentrations. The two types of ITC induced the expression of cyanide hydratase and of an ABC-transporter gene similar to BcAtrB from*Botrytis cinerea* ; benzyl-ITC also significantly affected the expression of a glutathione S-transferase homologue. Up-regulation of the expression of the ABC transporter gene by CAM was also observed. We now plan to study the expression patterns of these *Alternaria brassicicola* genes in planta using experimentally inoculated *Arabidopsis thaliana* plants.

340. Physiological and molecular responses of *Alternaria brassicicola* to exposure to host plant antimicrobial compounds. Adnane Sellam, Pascal Poupard, Philippe Simoneau UMR PaVé N°77, Faculté des Sciences, 2 Bd Lavoisier, 49045 Angers cedex, France

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341. Withdrawn

342. Withdrawn

343. Physiological and molecular responses of *Alternaria brassicicola to exposure to host plant antimicrobial compounds.* Adnane Sellam, Pascal Poupard, Philippe Simoneau. UMR PaVé N°77, Faculté des Sciences, 2 Bd Lavoisier, 49045 Angers cedex, France

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344. Dissection of Distinct Pathogenesis-related Processes in the Blast Fungus *Magnaporthe grisea*. Ane Sesma and Anne E. Osbourn. The Sainsbury Laboratory, John Innes Center, Colney Lane, Norwich NR4 7UH, U.K.

The blast fungus *Magnaporthe grisea* , which causes disease in a wide variety of grasses including rice, wheat and barley, has emerged as a paradigm system for investigation of foliar pathogenicity. This fungus undergoes a series of well-defined developmental steps during leaf infection, including the formation of elaborate penetration structures (appressoria). Remarkably, *M. grisea* can also infect roots. We have shown that it can undergo a different (and previously uncharacterised) set of programmed developmental events that are typical of root-infecting pathogens that can lead to systemic invasion and the development of classical disease symptoms on the aerial parts of the plant. However, the ability to infect roots does not depend on appressoria. In contrast, hyphal swellings resembling the simple penetration structure (hyphopodia) of root-infecting fungi are evident at infection sites. In addition, experiments with defined mutants defective in leaf infection confirm that there are key differences between the molecular determinants and signal transduction requirements for penetration of leaves and roots. Using cytological approaches (scanning electron microscopy and confocal microscopy) we are extending these studies to identify the step in which these mutants are unimpaired during root colonisation. We are also testing a collection of *M. grisea* wild-type and mutant strains on a range of grooved topographies with different chemical, hydrophobic and thickness properties to find *in vitro* conditions for hyphopodium formation.

345. *FSR1*, a putative WD40 repeat homolog in *Fusarium verticillioides*, plays a role in maize stalk rot virulence. W. B. Shim, H. W. Wilkinson, U. S. Sagaram, and Y. E. Choi. Department of Plant Pathology and Microbiology, Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX77843-2132

F. verticillioides (teleomorph *Gibberella moniliformis*) causes stalk rot of maize, the most prevalent disease in all maize-growing regions. Using a forward genetics approach, we have identified a locus, *FSR1*, implicated in Fusarium stalk rot pathogensis; the *fsr1* mutant exhibits a loss of virulence and fails to cause rot in maize stalks. Sequencing of the disrupted locus revealed a 2718-bp ORF with two introns. Conceptual translation resulted in a 824-amino acid polypeptide with 60% identity to *Sordaria macrospora* Pro11, which is a multimodular protein with two distinct functional domains known to be involved in protein-protein interactions: coiled-coil motif and seven-WD40 repeats. Pro11 plays an important role in cell differentiation, particularly sexual development in *S. macrospora*. Significantly, Pro11 homologs are highly conserved and found in a number of mammalian systems, filamentous fungi and *Drosophila*, but not in yeasts and plants. While the role of Pro11 homologs in pathogenic fungi has not been previously characterized, our data indicate that *F. verticillioides FSR1* is involved in fungal virulence. Additional studies are in progress to characterize the relationships among genes in this gene family and to assess whether *FSR1* is a key molecular component in a putative signaling pathway regulating stalk rot virulence.

346. Dissecting the role of signal transduction in *Stagonospora nodorum* during infection on wheat. Peter S. Solomon[1], Kar-Chun Tan[1], Ormonde D.C. Waters[1], A. Harvey Millar[2], Richard P. Oliver[1]. [1]Australian Centre for Necrotrophic Fungal Pathogens, DHS, SABC, Murdoch University, Perth 6150, WA, Australia. [2] University of Western Australia, School Biomedical and Chemical Sciences, 35 Stirling Highway, Perth 6009, WA Australia.

Stagonospora nodorum is a fungal pathogen of wheat that has the potential to severely affect the economic viability of Australia's wheat crop. Whilst having been identified as serious wheat disease for many years, the molecular understanding of how the fungus and its host interact is poorly understood. A reverse genetics approach has been adopted to find genes involved in pathogenicity focusing predominantly on signalling and primary metabolism pathways. To better understand the role of signal transduction in pathogenicity, genes encoding a Gα subunit (*Gna1*) from the cAMP pathway and a MAP kinase (*Mak2*) from the MAP kinase pathway were characterised by gene disruption. Fungal strains developed with mutations in either gene were severely reduced pathogenicity and both were unable to sporulate. Infection assays revealed the *mak2* strain was virtually non-pathogenic whilst the *gna1* strain appeared weakly pathogenic and was able to penetrate the leaf through natural openings. Both mutations also revealed severe phenotypic alterations specific for each mutation including melanin synthesis deficiencies, sensitivity to osmotic stress, reduced protease production/secretion. These results suggest that these key signalling genes control processes downstream which are required for pathogenicity. To determine what these processes are, a proteomics approach has been adopted. A comparison of the wild-type, *gna1* and *mak2* proteomes has identified proteins that are controlled by either *gna1*, *mak2* or both. A detailed analysis of these key signalling mutants and a synopsis of the proteomics will be presented.

347. Three novel retrotransposons from *Magnaporthe grisea* mini-chromosome. Teruo Sone, *Akio Oguchi, *Hisashi Kikuchi, **Akihiro Senoh, **Satoshi Nakagawa and Fusao Tomita. (Grad. Sch. Agr., Hokkaido Univ., Sapporo JAPAN * NITE, Tokyo, JAPAN **XANAGEN, Co. Tokyo Japan)

Most of *Magnaporthe grisea* rice-pathogenic field isolates have mini-chromosomes. These mini-chromosomes are less than 2 Mb in size and known to be related to fertility. Strain 70-15, used in the genome sequence project of *M. grisea* lacks mini-chromosomes. Thus we attempted to sequence the mini-chromosome of this fungus. A shotgun library of 1.6 Mb mini-chromosome from the strain 9439009, Japanese field isolate was constructed. Sequencing of both ends of inserts were completed for 28800 clones of the library, but assemble was not successful even by the masking of known transposable elements. One of the estimated reasons for this failure was the occurrence of many unknown repetitive elements. Therefore we manually extracted 71 repetitive sequences with consed program. Three novel retrotransposon-like sequences were identified from these repetitive sequences.
Further characterization of these sequences was attempted. Two of these elements, named *Inago 1* and *Inago 2*, were 58.8 % homologus in amino-acid sequence of pol gene product, showing the structural similarity to *Gypsy*/*Ty3* family of retrotransposons. Interestingly, LTR (Long terminal repeat) of these elements were known repetitive elements of *M. grisea*, MGR608 and REP1, respectively. Other one element, named *Swarm*, indicated structural similarity with *Copia*/*Ty1* family of retrotransposons. LTR of *Swarm* exhibited homology with IGS (intergenic spacer sequence) of rDNA.

348. Genetic Evidence for a Role of *Phytophthora infestans* protease inhibitors in disease. Jing Song, Nicolas Champouret, Joe Win, Miaoying Tian, Sophien Kamoun. Department of Plant Pathology, The Ohio State University-OARDC, Wooster, OH 44691

The oomycete *Phytophthora infestans* causes late blight, a reemerging and ravaging disease of potato and tomato. *Phytophthora infestans* has evolved 18 extracellular protease inhibitor genes belonging to two major structural classes: (i) Kazal-like serine protease inhibitors (EPI1-14) and (ii) cystatin-like cysteine protease inhibitors (EPIC1-4). Biochemical evidence suggests that some of the protease inhibitors target apoplastic proteases from the host plant tomato. EPI1 and EPI10 inhibit and interact with the subtilisin-like protease p69B, whereas EPIC2 interacts with the papain-like cysteine protease PIP1. To complement the biochemical studies, we first carried out stable DNA transformation of protoplasts of *Phytophthora infestans* using lipofectin, polyethylene glycol and CaCl2. We used the Gateway technology to generate transformation constructs in which the gene of interest is bordered by the oomycete *ham34* promoter and terminator, followed by the bacterial resistance gene G418 (Geneticin) fused to *hsp70* promoter and terminator. Both sense and antisense constructs of *epi* genes were used. We also used RNA interference (RNAi) triggered by dsRNA to silence *epi*genes. Preliminary evidence suggests that alteration of *epi1* expression resulted in altered virulence on tomato. These studies will lead to a better understanding of the role of protease inhibitors in disease progression.

349. Coordinate regulation of metabolic genes and identification of a virulence gene expression cluster in *Blumeria graminis*. M Both, M Czukai, PD Spanu. Imperial College London and Syngenta Ltd

We used cDNA microarrays to measure the expression of 2027 unigenes of *Blumeria graminis* f. sp. *hordei*, an obligate biotrophic pathogen of barley. The relative expression of these genes was followed throughout the normal course of *B. graminis* development from the ungerminated conidium, its early germination stages (4, 8 and 15hpi) to the proliferation on the host surface and in barley epidermis containing haustoria (3 and 5 dpi). Global correlation analysis showed that there was a marked shift in gene expression between the pre- and post-penetrative stages. This was due primarily to large scale changes in the metabolism of the fungus as it progressed though its development. For example, genes devoted to translation were significantly up-regulated when the fungus proliferates in the post-penetrative stages. Some of the genes encoding enzymes on common primary metabolic pathways showed striking coordinate expression. Thus, "glycolytic transcripts" increased significantly immediately after germination, then decreased and increased again in the samples containing haustoria. Glycogen degradation transcripts were abundant at early stages (4 and 8 hpi) and later decreased; this was matched by a significant peak in glycogen branching transcript when conidia are formed. Many lipid degradation transcripts levels were high at the early stages and decrease to low levels thereafter. The AdoMet cycle transcripts were most abundant in the proliferating and sporulating epiphytic mycelium.
There are a number of *B. graminis* cDNAs on our microarrays that are homologous to genes, such as *cap20*, known to affect pathogenicity in other fungi. Expression cluster analysis identified genes whose expression correlated with a *cap20* homolog. One such expression cluster included a striking proportion of genes that are associated to pathogenicity. Applying the "guilt-by-association" principle identified a number of cDNAs that are potential candidates for pathogenicity genes in *B. graminis*.

350. A gene involved in modification of transfer RNA is required for fungal pathogenicity and stress tolerance of *Colletotrichum lagenarium*. Yoshitaka Takano[1], Naoyuki Takayanagi[1], Hiroyuki Hori[2], Yoshiho Ikeuchi[3], Tsutomu Suzuki[3], Akiko Kimura[1], and Tetsuro Okuno[1]. [1]Kyoto University, Japan. [2]Ehime University, Japan. [3]University of Tokyo, Japan.

7-methylguanosine (m^7G) modification of tRNA occurs widely in eukaryotes and prokaryotes, although there is little information about its biological roles. We report here that a gene involved in m^7G modification of tRNA is required for pathogenicity in *Colletotrichum lagenarium* that causes cucumber anthracnose. The pathogenicity mutant KE51 of *C. lagenarium* was identified by insertional mutagenesis. Analysis of KE51 identified a plasmid-tagged gene *APH1*. The *aph1* deletion mutants failed to infect host plants. Aph1 showed strong similarity to *Saccharomyces cerevisiae* Trm8 involved in m^7G modification of tRNA. RNA from the *aph1* mutant has much reduced m^7G compared with the wild type, indicating that *APH1* is required for m^7G methyltransferase activity. Appressoria formed by the mutants failed to develop penetration hyphae into the host plant, suggesting requirement of *APH1* for appressorium function for plant infection. Interestingly, the *aph1* mutants increased sensitivity to several stresses such as H_2O_2 treatment, implying that penetration defects of the *aph1* mutant depend on a failure to protect itself against plant defense systems. Consistent with this, heat shock treatment on the host plants enabled the *aph1* mutant to penetrate into them. These data suggest a possibility that the fungal pathogen require Aph1-mediated tRNA modification to overcome plant defense.

351. Reactive oxygen species generated by a fungal NADPH oxidase regulate hyphal differentiation and growth in *Epichloe/N festucae*, a mutualistic symbiont of temperate grasses. Aiko Tanaka[1], Michael Christensen[2] and Barry Scott[1]. [1]Institute of Molecular BioSciences, Massey University, Private Bag 11 222, Palmerston North, New Zealand. [2]AgResearch Limited, Grasslands Research Centre, Private Bag 11 008, Palmerston North, New Zealand.

Epichloe /Neotyphodium endophytes are a group of clavicipitaceous fungi (Clavicipitaceae, Ascomycota) that form symbiotic associations (symbiota) with temperate grasses of the sub-family Pooideae. The hyphae of these endophytes grow systemically through the intercellular spaces of the leaf as seldom branched linear strands and the host remains symptomless. Plasmid insertional mutagenesis was used to identify genes of *E. festucae* responsible for maintaining the mutualistic association. From a screen of 220 transformants, one mutant FR2 was identified that causes severe stunting of the grass host characterized by an increase in tiller number, and in most cases eventual death of the host. Associations containing FR2 show hyphal hyper-branching and a dramatic increase in fungal biomass, although the growth of FR2 was normal in culture. The genomic DNA flanking the single-copy plasmid insertion in FR2 was isolated by plasmid rescue. Sequence analysis of the recovered fragment showed that the plasmid was inserted into the coding region of a NADPH oxidase gene (designated *EfnoxA*). *Nox* genes are homologous to *gp91phox*, the glycosylated transmembrane subunit of the mammalian NADPH oxidase that catalyses production of reactive oxygen species (ROS). Complementation and deletion analysis confirmed that the phenotype of FR2 is caused by disruption of *EfnoxA*. Using the cerium chloride method ROS accumulation was detected cytochemically near cell walls of fungal hyphae in wild-type associations. These results demonstrate that ROS production by the endophyte NADPH oxidase is crucial in maintaining a mutualistic association between the endophyte and its grass host.

352. Molecular characterization of the pea pathogenicity (*PEP*) gene cluster of *Neocosmospora boniensis* and other *Neocosmospora* species. Esteban D. Temporini, Dai Tsuchiya, and Hans D. VanEtten. Division of Plant Pathology & Microbiology, University of Arizona, Tucson AZ.

In 1989, Udagawa and colleagues (SYDOWIA 41: 349-359) described a new species belonging to the genus *Neocosmospora: N. boniensis*. Although *Neocosmospora* species have been described as pathogens of several plant species, there are no reports on the literature showing this group of fungi as naturally occurring pea-pathogens. We have recently shown that *N. boniensis* contains homologs of the genes present in the pea pathogenicity (*PEP*) cluster of *Nectria haematococca* MPVI, which are required by *N. haematococca* to cause disease on pea. Moreover, we have shown that the *PEP* homologs are also clustered in *N. boniensis* and that this species is capable of causing disease on pea. In this work, DNA fiber-FISH experiments were used to analyze the physical organization of the homologs in the *N. boniensis PEP* cluster. The results indicate that several rearrangements have occurred in the *N. boniensis PEP* cluster, compared to the *N. haematococca PEP* cluster. These rearrangements have been confirmed by determining the nucleotide sequence of this region, which shows that transposable elements may have played a role in shaping the *N. boniensis PEP* cluster. This analysis has also revealed that the DNA homology between the *PEP* genes of *N. boniensis* and *N. haematococca* is very high (around 95% identity). We also show here that *PEP* homologs are present in other species of *Neocosmospora* and that these species are capable of causing disease on pea.

353. Dissection of the transcriptome of *Phytophthora sojae* under oxidative stress: Source of pathogenicity factors? Trudy Torto-Alalibo, Dianjing Guo, Regina Hanlon, Hua Li and Brett Tyler. Virginia Bioinformatics Institute, Virginia Tech, Blacksburg VA, 24061

One of the hallmarks of plant defense responses against pathogens is the generation of reactive oxygen species (ROS) in a process referred to as oxidative burst. Extensive studies have been conducted to investigate the role of ROS in plant defense; however, little has been done to determine the role of the pathogen under oxidative stress. Recent studies have shown that the production of ROS in *Botrytis cinerea*, a necrotroph, leads to increased levels of ROS production in the plant, which causes death of plant tissue. Subsequently, increase in fungal growth is observed. Alternatively, the biotrophic fungus, *Claviceps purpurea*, produces scavenging enzymes to protect itself from oxidative stress. Studies have shown that some key genes such as catalase and the bZIP transcription factor identified when pathogens are under oxidative stress contribute to pathogenicity. *Phytophthora sojae* is a hemibiotroph, which causes root rot to soybean resulting in billions of dollars in losses to farmers annually. Responses of either the pathogen or the plant at the point of interaction (interactome) are not well studied. Genes differentially expressed under oxidative stress by *P. sojae* may have a role in the modulation of host defense reactions. We used the technology of microarray, which helps to look at a global expression of genes under a particular condition to study the transcriptome of *P. sojae* under oxidative stress. *P. sojae* mycelium was grown *in vitro* and subjected to 0.3mM cumene hydroperoxide. Samples were taken at six time points viz. 0, 5 mins , 15 mins, 30 mins, 1hr and 2hrs. Results from the data generated from the microarray will be presented.

354. Investigating the interaction between *Magnaporthe grisea* and rice through a comprehensive timecourse experiment using microarray analysis. Sara L. Tucker[1], Nicole Donofrio[2], Ralph Dean[2], David A. Henderson[1] and Marc J. Orbach[1]. [1]University of Arizona, Tucson, AZ 85721. [2]North Carolina State University, Raleigh, NC 27695, USA.

Magnaporthe grisea is responsible for blast disease of rice. We are using this system to identify fungal and plant genes that are differentially expressed during infection and growth of the fungus in planta. To study global changes in gene expression, an interactions microarray was developed in collaboration with Agilent Technologies, which contains identifiers for 13666 *M. grisea* genes and 7137 *Oryza sativa* genes. We are characterising pathogen and host gene expression in a compatible interaction over a six-day period following infection. Hybridisations were carried out using a loop design to best compare gene expression at several time points during the infection cycle. This also allows the comparison of expression at additional time points after analysis of the initial data. The initial loop compares gene expression of uninoculated plants with infected plants at nine time points over a six-day period following infection. Additional infected tissue was collected up to 10 days post infection for potential future analysis. The data generated from this experiment has allowed us to cluster genes with similar expression patterns over the infection cycle and provisionally assign them to certain developmental stages of the rice/*M. grisea* interaction. The data is currently being validated using Real Time-PCR and these results will be presented.

355. The role of ROS generating and scavenging systems in host-pathogen interaction. N. Segmüller, S. Giesbert, E. Nathues, P. Tudzynski. Institut für Botanik, Westf. Wilhelms-Universität, Schlossgarten 3, 48149 Münster, Germany

Reactive oxygen species (ROS) are normal cellular components and recently have been shown to be involved in differentiation processes in several fungi. Since the generation of ROS is a major defense reaction of plants (“oxidative burst”), in fungi/plant interactions ROS are produced by both partners. We study the role of ROS scavenging systems (catalases, etc.) and ROS generating systems (toxins, NADPH oxidases) in pathogenic processes in two interaction systems with different strategy: the biotrophic rye pathogen, *Claviceps purpurea*, and the necrotrophic grey mould, *Botrytis cinerea*. We can show that the latter efficiently reduces ROS formation *in planta* [1], wherease *B. cinerea*, uses ROS as “weapon”. In both systems components of the stress-activated MAPK cascade are investigated.

1 Nathues E, Joshi S, Tenberge KB, von den Driesch M, Oeser B, Bäumer N, Mihlan M, Tudzynski P (2004) CPTF1, a CREB-like transciption factor is involved in the oxidative stress response in the phytopathogen *Claviceps purpurea*, and modulates ROS level in its host Secale cereale. Mol Plant Microbe-Interact 17: 383-393.

356. The G alpha subunit BCG1 of *Botrytis cinerea* controls at least two signalling cascades in the interaction with plants. Julia Schumacher, Christian Schulze Gronover, and Bettina Tudzynski, Institut für Botanik, Westfälische-Wilhelms-Universität Münster, Schlossgarten 3, D-48149 Münster, Germany

The G-alpha subunit BCG1 plays an important role during the infection of plants by *B. cinerea*. *bcg1* mutants are able to penetrate host tissue and to produce small primary lesions. However, the mutants completely stop invasion of plant tissue at this stage never producing spreading lesions. SSH was used to identify fungal genes which are up-regulated *in planta* in the wild-type, but not expressed anymore in *bcg1* mutants. Among the differentially expressed genes we found those encoding proteases, enzymes involved in secondary metabolism, hexose transporters, and cell wall-degrading enzymes. To examine if the expression of the BCG1 target genes is also affected in the adenylate cyclase (BAC) mutant, we performed Northern blot analysis with RNA from bean leaves infected with the wild-type, *bcg1* or *bac* mutants. Unexpectedly, most of the BCG1-controlled genes are still expressed in the *bac* mutant *in planta*, suggesting that BCG1 is involved at least in one additional signalling cascade beside cAMP-depending pathway. Only four of the genes are indeed regulated by BCG1 and BAC. To find out whether the G-beta/gamma dimer controls the unknown signalling pathway, we cloned and characterized the G-beta subunit-encoding gene, *bcgb1*. Furthermore, GUS-reporter gene assays with different promoter fragments of a xylanase-encoding gene (cAMP-pathway) and a metalloprotease-encoding gene (yet unknown pathway) were performed in order to identify binding motifs for the corresponding transcription factors for 'yeast one-hybrid' assays.

357. Comparison of gene expression in trap cells and vegetative hyphae of the nematophagous fungus *Monacrosporium haptotylum*. Dag Ahrén[1,2], Margareta Tholander[1], Csaba Fekete[1], Balaji Rajashekar[1], Eva Friman[1], Tomas Johansson[1], and Anders Tunlid[1]. [1]Department of Microbial Ecology, Lund University, Lund, Sweden. [2]European Bioinformatic Institute, Hinxton, Cambridge, UK.

Nematode-trapping fungi enter the parasitic stage by developing specific morphological structures called traps. We have compared the global patterns of gene expression in traps and mycelium of the fungus *Monacrosporium haptotylum*. The trap of this fungus is a unicellular spherical structure called the knob, which develops on the apex of a hyphal branch. RNA was isolated from knobs and mycelium and hybridised to a cDNA array containing probes of 2,822 EST clones of *M. haptotylum*. Despite the fact that the knobs and mycelium were grown in the same medium, there were substantial differences in the patterns of genes expressed in the two cell types. In total, 23.3 % (657 of 2,822) of the putative genes were differentially expressed in knobs versus mycelium. Several of these genes displayed sequence similarities to genes known to be involved in regulating morphogenesis and cell polarity in fungi. Several homologues to genes involved in stress response, protein synthesis and protein degradation, transcription, and carbon metabolism were also differentially expressed. A number of the genes that were differentially expressed in trap cells are also known to be regulated during the development of infection structures in plant pathogenic fungi.

358. The transcription-associated proteins of *Fusarium graminearum* identified by sequence clustering and profile analyses. Richard Coulson[1], Martin Urban[2], John Antoniw[2], and Kim Hammond-Kosack[2]. [1]European Bioinformatics Institute, Wellcome Trust Genome Campus, Cambridge, CB10 1SD, UK and [2]Rothamsted Research, Herts, AL5 2JQ, UK

The trichothecene mycotoxin producing Ascomycete fungus *Fusarium graminearum* causes ear blight disease of small grain cereals. Infections lower grain quality and safety, and are of increasing global concern. In 2003, its genome was sequenced to ~10 × coverage by the Broad Institute (http://www.broad.mit.edu/annotation/fungi/fusarium). As part of the global initiative to complete the manual annotation of the genome, we have explored in depth *F. graminearum* sequences involved with the transcriptional process. Eukaryotic transcription is a highly regulated process involving interactions between large numbers of proteins, exhibiting a high degree of taxon-specificity. To identify transcription-associated proteins (TAPs), the genome was queried with a reference set of TAPs, extracted from the protein sequence databases via keyword searches (Coulson & Ouzounis (2003) Nucleic Acids Research 31, 653-660). The TRIBE-MCL algorithm was employed to detect TAP families in *F. graminearum*, in addition to those present in six model organism species: *Schizosaccharomyces pombe, Saccharomyces cerevisiae, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster*, and *Mus musculus.* Four plant pathogens *Ashbya gossypii, Magnaporthe grisea, Aspergillus oryzae, Ustilago maydis,* two human pathogens, *Candida albicans* and *Aspergillus fumigatus* and two free living saprophytes, *Aspergillus nidulans* and *Neurospora crassa* were also included in the study. The findings from this TAP-TRIBE analysis, and a second complementary approach utilising profile-hidden Markov models of domains present in well-characterised transcriptional regulators, will be presented. Currently, we are exploring the physical distribution of each TAP gene amongst the four *F. graminearum* chromosomes.

359. The *Fusarium oxysporum* avirulence protein Six1 is required for full virulence, and is expressed early during infection of tomato. H. Charlotte van der Does, Michiel Meijer, Ben J.C. Cornelissen and Martijn Rep. University of Amsterdam, Swammerdam Institute for Life Sciences, Plant Pathology, Kruislaan 318, 1098 SM Amsterdam, The Netherlands

Fusarium oxysporum f.sp. *lycopersici* (Fol), is a soil inhabiting fungus that can infect tomato plants via the roots and colonize the xylem. In xylem vessels Fol secretes a 12 kD, cysteine rich protein (Six1) which appears to be derived from a 30 kD precursor through proteolytic processing by fungal proteases. The *SIX1* gene is required for resistance of tomato plants carrying the *I-3* resistance gene against Fol, but is also required for full pathogenicity of Fol, especially on older (4 week old) tomato plants. *SIX1* expression is highly induced *in planta*, compared to *in vitro* conditions. Expression starts already before the 4th infection day, when no disease symptoms are visible yet, and deminishes at later stages of infection, when plants show severe disease symptoms. To monitor expression in more detail, we have constructed *SIX1* promoter-*GFP* fusion genes, which have been placed at the native *SIX1* locus. In addition, to search for proteins interacting with Six1, we have produced the Six1 protein with an internal His-tag in *Pichia pastoris. P. pastoris* appears to process the 30 kD Six1 precursor in the same way as Fol does.

360. Characterization and functional analysis of a *Botrytis cinerea* aspartic proteinase gene family. Arjen ten Have, Ester Dekkers, John Kay, Lowri H. Phylip and Jan A.L. van Kan. Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands

Botrytis cinerea secretes aspartic proteinase (AP) activity in axenic cultures. No cysteine, serine or metalloproteinase activity was detected. Proteinase activity was higher in cultures containing BSA or wheat germ extract as compared to minimal medium. AP was also the only proteinase activity in fluid obtained from *B. cinerea*-infected tissue of apple, pepper, tomato and zucchini. Five *B. cinerea* genes encoding an AP were cloned and named *Bcap*1-5. Especially BcAP1 has novel characteristics. A phylogenetic analysis was performed comprising sequences originating from different kingdoms. BcAP1 and BcAP5 did not cluster in a bootstrap supported clade. BcAP2 clusters with vacuolar APs. BcAP3 and BcAP4 cluster with secreted APs in a clade that also contains GPI-anchored proteinases from *S. cerevisiae* and *C. albicans*. All five *Bcap* genes are expressed in liquid cultures. Transcript levels of all genes except *Bcap5* are subject to glucose and peptone repression. All five *Bcap* genes are expressed in infected tissue, indicating that at least part of the AP activity in planta originated from the pathogen. Previous research showed that pepstatin could inhibit infection by *B. cinerea*, suggesting an important role for APs in the infection process. We analysed the role of APs by gene replacement. Mutants were generated in all five genes separately, as well as in combinations of two *Bcap* genes. None of the mutants displayed a reduction in virulence or in secreted AP activity.

361. Withdrawn

362. Growth and colonization of *Colletotrichum graminicola* inside corn stalk tissues. C. M. Venard and L. Vaillancourt. University of Kentucky, Plant Pathology Dpt.

Anthracnose disease, caused by the fungus *C. graminicola*, is one of the greatest threats to corn production in the U.S. today. The pathogen can attack any part of the plant at any time during the growing season. The most common symptoms are those affecting leaves (blight) and stalk (rot). Anthracnose stalk rot has the greatest economic impact on harvestable yield, but this phase of the disease is poorly understood. We developed an inoculation method that reproduces ASR field symptoms in the greenhouse and used a cytological approach to study pathogen development inside stalk tissues. Fungal strains expressing green fluorescent markers were used to inoculate a highly susceptible sweet corn hybrid. We observed the formation of a highly reproducible, highly organized hole-like lesion under the rind that we called an infection chamber. Necrotic tissues, on which the fungus produces falcate spores in acervuli, surround the hole. The pathogen also colonized the necrotic tissues at the margins of the infection chamber, where it was observed to produce oval conidia inside the parenchyma cells. The fungus was observed to move through the stalk primarily as mycelium inside fibers under the rind, and via the vascular bundles that were damaged by the expansion of the initial lesion. Secondary lesions were initiated from these colonized vascular tissues. When the fungus reached the node, its progression was stopped until the tissues in the plexus collapsed. The fungus then progressed across the node into the upper internode, where it initiated new lesions.

363. FGL1, a secreted lipase of *Fusarium graminearum* is a novel virulence factor during infection of cereals. Christian A. Voigt, Wilhelm Schaefer, and Siegfried Salomon. Department of Molecular Phytopathology and Genetics, University of Hamburg, Germany.

Fungal pathogens have evolved a number of different strategies to infect and colonize host plants. A lot of fungal pathogens secrete various extracellular enzymes like xylanases, pectinases, cutinases, and proteinases which are supposed to be involved in host infection. We could detect, clone, and characterize a secreted lipase (FGL1) of *F. graminearum*. The functional identity of the lipase was examined by heterologous gene expression in *Pichia pastoris*.
In planta, FGL1 transcripts were already detected one day after inoculation of wheat spikes. To evaluate the role of FGL1 during the infection process we created lipase deficient mutants by gene disruption and compared them to the wild type strain. Gene disruption of FGL1 resulted in a significantly reduced extracellular lipolytic activity of the mutants. After infection of wheat spikes, the FGL1 deficient strains showed a drastically reduced virulence. In contrast to *F. graminearum* wild type infected wheat spikes, FGL1 deficient strains were unable to colonize the rachis of the spike. Infections of spikes were therefore restricted to the point of inoculation. Additionally, maize ears inoculated with *F. graminearum* wild type conidia are fully infected. In contrast, the maize ears develop normally and showed minor disease symptoms when inoculated with FGL1 deficient strains. Our data are the first molecular proof that a secreted lipase is a major virulence factor of a fungal pathogen.

364. Genetics of avirulence in *Mycosphaerella graminicola*, the wheat septoria leaf blotch fungus. Sarah B. Ware[1] and Gert H.J. Kema[1]. [1]Plant Research International, Wageningen, The Netherlands.

To elucidate the genetics of avirulence in *Mycosphaerella graminicola*, 163 progeny of an *in vivo* cross between a Dutch bread wheat-derived isolate, IPO323, and an Algerian durum wheat-derived isolate, IPO95052, are currently undergoing pathogenicity testing on a total of seven cultivars of bread and durum wheat. Preliminary results show many phenotypic recombinants and very few parental phenotypes. Although IPO323 did not infect any of the durum wheat cultivars and IPO95052 did not infect any of the bread wheat cultivars, 25% of progeny were able to infect both. Neither IPO323 nor IPO95052 were able to infect the bread wheat cultivar Shafir, but 10% of the progeny were able to produce pycnidia on this cultivar. Ten percent of progeny infected only bread wheat cultivars (at least one of four) and 25% of progney infected only durum wheat cultivars (at least one of three). Thirty percent of progeny were not able to infect any of the seven cultivars tested. Certainly, there are many genes involved in avirulence in *M. graminicola*. The genetics of avirulence on bread wheat and durum wheat cultivars in *M. graminicola* is apparently under simple genetic control. Furthermore, because a virulent isolate and an avirulent isolate can cross and produce viable offspring, it can be concluded that for *M. graminicola*, a resistant host does not necessarily stop gene flow.

365. Cercosporin is a virulence factor in the infection of sugar beet by *Cercospora beticola*. John J.Weiland[1], Kuang-Ren Chung[2] and Jeffrey C. Suttle[1]. [1]Sugarbeet and Potato Research, USDA-ARS-RRVARC, Fargo, ND, 58105 and [2]University of Florida, Lake Alfred Research Station, Lake Alfred, FL, 33850

A portion of the CTB gene encoding an enzyme in the cercosporin biosynthetic pathway of Cercospora species was cloned from C. beticola. Using a transformation vector harboring these sequences, the endogenous CTB gene in C. beticola was disrupted by homologous recombination. Southern and northern blot analysis confirmed that transformants lacking secretion of the red pigmented cercosporin toxin possessed a disrupted CTB gene and lacked vector integration events in other regions of the genome. Five CTB mutants (ctb-delta2, -delta3, -delta21, -delta23, and -delta24) examined produced reduced cercosporin as compared to parent C. beticola isolate 303B. All isolates possessed radial growth rates indistinguishable from 303B and retained the ability to sporulate in culture. Inoculation of sugar beet plants with the five mutants induced a lower number of leaf spot lesions that expanded at a reduced rate as compared to that produced by isolate 303B. Cercosporin accumulation in leaves infected with the mutants as compared to that from leaves infected by the parent will be presented. The data indicate that cercosporin is a virulence factor in the infection of sugar beet by C. beticola.

366. Functional analysis of *CLSTE12*, an *STE12*-like gene isolated from the bean pathogen *Colletotrichum lindemuthianum*. Wong Sak Hoi J., Herbert C., Dumas B. UMR 5546 CNRS-Université Paul Sabatier, Pôle de Biotechnologie Végétale, 24 chemin de Borde-Rouge, 31326 Castanet-Tolosan, France
STE12 is a transcription factor that regulates yeast mating and invasive growth. This factor is activated by a MAPKinase cascade involving KSS1. In many phytopathogenic fungi, orthologue genes of *KSS1* are essential for infection, suggesting a role for STE12-like factors in pathogenicity. We isolated an *STE12*-like gene (*CLSTE12*) from *Colletotrichum lindemuthianum*, the causal agent of bean anthracnose. This gene encodes a 705 amino acid protein containing a homeodomain at the N-terminal region and two Cys2His2 zinc fingers at the C-terminal region. The homeodomain is present in all the proteins from the STE12 family ever identified, whereas zinc fingers are specific to filamentous fungi. We produced truncated versions of CLSTE12 in *Escherichia coli*, including both, one or no zinc fingers. Gel shift experiments revealed that the protein was able to bind to the yeast STE12 recognition site despite the deletions, indicating that the zinc fingers are not essential for DNA-binding. To define the function of CLSTE12, *clste12* mutants were obtained by targeted gene disruption. Saprophytic growth of the mutants was indistinguishable from that of the wild type strain. These mutants were able to form appressoria, but did not produce anthracnose lesions on bean leaves, indicating that *CLSTE12* is essential for the pathogenicity of *C. lindemuthianum*.

367. G protein signaling mediates developmental processes and pathogenesis of *Alternaria alternata*. Daisuke Yamagishi, Hiroshi Otani and Motoichiro Kodama. Plant Pathology Lab, Dept Agriculture, Tottori University, Tottori 680-8553, Japan.

To investigate possible signal transduction pathways that might be related to basic compatibility of toxigenic and necrotrophic *A. alternata* pathogens, we have cloned and characterized a Ga gene from the apple pathotype of *A. alternata*. In addition, we observed whether the gene controls the production of secondary metabolites, particularly host-specific toxins responsible for host-specific pathogenicity or vilrulence of the pathogen. A PCR-based approach was employed to isolate a Ga gene (designated *AGA1*) from the apple pathotype. *AGA1* shows a high degree of similarity to other fungal Ga genes. Targeted disruption of *AGA1* gave rise to mutants that differed in colony morphology, compared to the wild type, and sporulation of the mutants dramatically decreased in vitro. In addition, the mutants showed decreased conidial length and width, and few longitudinal septa were formed. The development of infection structures on cellulose membranes was also compared between the wild type and the mutants. The conidia of wild type, an ectopic transformant and the mutants showed equal germination. However, wild type germ tube formed readily from different points around the conidia, grew randomly and were often branched, whereas those of the mutants formed only at one or both ends of the conidia and tended to grow in straight paths. In addition, targeted disruption of *AGA1* resulted in reduction of pathogenicity on apple leaves, although the mutant produced host-specific AM-toxins at levels similar to the wild type strain. Measurement of the intracellular cAMP levels of the mutant indicated that it was consistently higher than that of the wild type, indicating that *AGA1* negatively regulates cAMP levels similar to mammalian Gai systems. These results indicate that the signal transduction pathway represented by *AGA1* appears to be involved in developmental pathways leading to sporulation and pathogenesis of *A. alternata*

368. Molecular characterization of yeast Hsp40 homologues MHF17 and MHF23 from *Magnaporthe grisea*. Mihwa Yi and Yong-Hwan Lee. School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea

Magnaporthe grisea is a causing agent of rice blast disease considered as the most serious concern in rice growing areas world wide. *M. grisea* is a model plant pathogenic fungus with molecular genetic tractability and released genome sequences. Relied on recently developing bioinformatics tools, we discovered 26 putative HSP40s including conserved J domain in *M. grisea* genome database. To elucidate the functions of HSP40s in *M. grisea*, we preferentially determined to characterize two unique HSP40s, named as MHF17 and MHF23. MHF23, type II HSP40, is yeast Sis1 homologue, which is essential and function on initial translation stage. MHF17 has no homologue in *S. cerevisiae*, and belongs to type III Hsp40 family. The expression of *MHF17* and *MHF23* under heat shock was down regulated and no changes were detected in cold shock condition. *MHF17* and *MHF23* deletion mutants were generated by *Agrobacterium tumefaciens*-mediated transformation, which has well established as high efficient manner adopting positive negative selection strategy. *Mhf17* mutant showed sectoring morphology instead of radial growth on all solid media tested, most severe on oatmeal agar media, and reduction in asexual sporulation. Abilities on germination, appressorium formation, and pathogenicity on rice were not affected, and the defects on conidiation and radial growth on solid media were recovered with insertion of *MHF17* ectopically. *Mhf23* mutant undergoes germination and appressorium formation normally, and fully pathogenic on susceptible rice cultivar, and further characterization of phenotypes is in progress.

369. Functional genomics of *Phytophthora infestans* effectors of plant disease. Thirumala-Devi Kanneganti, Carolyn Young, Jorunn Bos, Joe Win and Sophien Kamoun. Department of Plant Pathology, The Ohio State University-OARDC, Wooster, OH 44691, USA

The oomycete *Phytophthora infestans* is a devastating pathogen of potato and tomato. During infection, *P. infestans* secretes a diverse array of effector proteins that manipulate host processes, leading to virulence. We hypothesize that *P. infestans* secretes two classes of effectors, one that is secreted into the plant apoplast and another that is translocated into the plant cell. Our goal is to understand the molecular mechanisms underlying *P. infestans*-plant interactions. For this purpose, computational data mining tools and robust high throughput functional assays were combined to identify candidate effector genes. We have selected ~200 full length cDNAs encoding extracellular proteins that fulfilled at least two of the following criteria: (1) up-regulated during infection; (2) conserved between *Phytophthora* and saprophytic/plant pathogenic fungi; (3) contain a nuclear localization signal; (4) possess R-dEER, a highly conserved novel amino acid motif present in virulence/avirulence proteins from three different oomycetes; (5) polymorphic between *P. infestans* strains. Single candidate genes were expressed *in planta* using virus vectors to identify genes that trigger cellular and molecular responses in plant cells. We aim to support our functional data with expression profiling and subcellular localization experiments. This research will provide the basis towards understanding *Phytophthora* effector gene function and will establish functional connections between *P. Infestans* effectors and plant processes.

370. Optimizing gene silencing and overexpression in *Phytophthora infestans* using the *PiBzp1* transcription factor, a gene required for appressorium formation and zoospore motility. Christina A. Bormann, Flavio A. Blanco, and Howard S. Judelson. Department of Plant Pathology, University of California, Riverside 92521 USA

Two priorities in studies of *Phytophthora* are identifying genes important in the disease cycles of these destructive pathogens, and improving methods for manipulating genes in transformants. For example, gene silencing or overexpression provides ways to characterize the role of genes in pathogenesis and validate their utility as targets for chemical inhibitors (i.e. fungicides). To help achieve these goals, we have been studying interactors of protein kinases induced during the spore cycle of *P. infestans*, the cause of late blight of potato and tomato. An interactor of a zoosporogenesis-induced kinase was found to encode a bZIP transcription factor, and experiments to misregulate the bZIP gene (*PiBzp1*) were initiated to test its role. Silenced transformants exhibited abnormal swimming behavior, failed to make appressoria, and were nonpathogenic. Using the bZIP gene as a model, optimal methods for both gene silencing and overexpression are now being identified. Constructs containing the bZIP coding region in sense or antisense directions between the *ham34* promotor and terminator, or the entire native gene, are being evaluated. Moreover, four different transformation techniques are being tested, involving protoplasts, electroporation, particle bombardment, and *Agrobacterium*. Preliminary results show that protoplast transformation results in more efficient silencing than electroporation.

371. Feedback-regulation of the Neurospora transcription factor White Collar Complex by the circadian clock protein Frequency. Tobias Schafmeier, Krisztina Káldi, Andrea Haase, Johanna Scholz and Michael Brunner. Biochemistry Center, University of Heidelberg (BZH), Im Neuenheimer Feld 328 Heidelberg D-69120, Germany

Circadian clocks organize temporal expression of large numbers of genes in many organisms. They are self-sustained cellular oscillators dependent on interconnected transcriptional/translational feedback-loops of clock proteins. FREQUENCY (FRQ) is a central component of the circadian clock of Neurospora. frq RNA is rhythmically expressed and FRQ feedback-regulates its own expression by inhibiting its transcriptional activator, the White Collar Complex (WCC). Here we have investigated the mechanism of negative feedback of FRQ on expression of its own RNA. We show that WCC is concentrated in the nucleus while the localization of FRQ is predominantly cytosolic. Furthermore, WCC is expressed in excess over FRQ in the nucleus and the bulk of WCC does not interact with FRQ. This suggests that WCC is not directly inactivated by complex formation with FRQ. Rather, we show that FRQ controls the phosphorylation state of WCC.

372. Nitrogen metabolite and nitrate signalling. Mark X Caddick[1], Igor Morozov[1], Greg Fitzgibbon[1], Ammar Razak[1], Joseph Strauss[2] and Meriel Jones[1]. [1] Biological Sciences, The University of Liverpool, UK. [2] Institut für Angewandte Genetik und Zellbiologie, BOKU-University, Vienna, Austria.

The GATA transcription factor AreA, which mediates nitrogen metabolite signalling in the filamentous fungus Aspergillus nidulans, is modulated by at least four distinct mechanisms. Our recent work has investigated two of these; the TOR kinase pathway and regulated transcript stability. In S. cerevisiae the TOR pathway acts via Ure2p to modulate the AreA orthologue Gln3p, and represents the predominant signalling mechanism. A. nidulans does not have a Ure2p orthologue but we have shown that the TOR pathway still contributes to nitrogen metabolite signalling through AreA. Previously we have shown that the areA transcript degrades rapidly in the presence of primary nitrogen sources (glutamine, ammonia) whilst remaining stable under conditions of nitrogen limitation, derepressing transcription of many genes involved in nitrogen metabolism. This 3' UTR-dependent degradation is preceded by rapid deadenylation. We have now demonstrated that nitrogen metabolites modulate the stability of further transcripts in these pathways, either accelerating or retarding decay. These include the niaD and niiA transcripts which are required for the reduction of nitrate to glutamine. Both transcripts degrade rapidly in the presence of glutamine and conversely, are stabilised by intracellular nitrate. Furthermore, when both glutamine and nitrate are present, these transcripts are stable, ensuring that nitrate and the toxic intermediate nitrite are always removed. Degradation of the niaD transcript is triggered by deadenylation, which is dependent on its 5' UTR. Nitrate inhibits poly(A) shortening even in the presence of glutamine. Therefore mRNA degradation, mediated through the poly(A) tail, is a fundamental part of the adaptive response to specific environmental signals.

373. Subcellular localization of velvet, a gene necessary for development and toxin production in *Aspegillus nidulans*. Suzanne M. Stinnett[1], Eduardo Espeso[2], and Ana Calvo[1]. [1]Northern Illinois University, Biological Sciences, Dekalb, Illinois. [2]Centro de Investigaciones Biológicas (C.S.I.C.), Microbiologia Molecular, Madrid, Spain

In *Aspergillus nidulans*, the velvet gene (*veA*) is required for morphological development and production of secondary metabolites. Previous studies have shown that deletion of *veA* results in a blockage in cleistothecia production. We have found that *veA* is also essential for the production of the mycotoxin sterigmatocystin and normal production of penicillin. Inspite of its importance, the *veA* mechanism of action remains unknown. In this study we investigated the subcellular localization of VeA to obtain clues about VeA molecular function. There is a conserved bipartite nuclear localization signal (NLS) motif present in the VeA N-terminal domain. With the goal of examining the functionality of this bipartite NLS, we generated *A. nidulans veA* fusion with the green fluorescent protein gene (*gfp*) and expressed them first in a yeast system. We observed that VeA was found in the nucleus and that such a transport is dependent on the alpha-importine protein SRP1. This interaction was also confirmed in a two-hybrid system assay. Currently we are examining the subcellular localization in *A. nidulans* and characterizing VeA NLS by expressing *gfp* fusions with the wild type *veA* allele and NLS *veA* mutant alleles.

374. Targeting stress-response genes for control of *Aspergillus* using antifungal natural compounds. Jong H. Kim[1], Bruce C. Campbell[1], Jiujiang Yu[2], Deepak Bhatnagar[2], and Thomas E. Cleveland[2]. [1]Plant Mycotoxin Research Unit, Western Regional Research Center, USDA-ARS, 800 Buchanan St., Albany, California 94710 USA; [2] Food and Feed Safety Unit, Southern Regional Research Center, 1100 Robert E. Lee Blvd, New Orleans, Louisiana, 70124 USA

Signal transduction and stress-response genes of fungal pathogens play important roles in pathogenesis. Many genes in *Saccharomyces cerevisiae*are interrelated to genes of many fungal pathogens. To discover stress-response genes critical for virulence, an *in silico* database of transduction/stress-response pathway genes of *Aspergillus flavus* was constructed based on orthologs of *S. cerevisiae*. Succesful functional complementation of an antioxidative stress gene from *A. flavus*, mitochondrial superoxide dismutase (*sodA*), in a *sod2*delta yeast mutant verified that *S. cerevisiae* could serve as a model system for functional genomics of *A. flavus*. Phenolics are released by plants during fungal infection and their detoxification is necessary for fungal pathogenesis. We developed a high throughput yeast bioassay to screen phenolics for potential antifungal or antiaflatoxigenic activities. Yeast genes affected by active compounds were identified, including a number of signal transduction and antioxidative stress response genes important to fungal tolerance. Then, compounds were used against *A. flavus*. A 100-fold synergism of strobilurin fungicides was achieved by targeting certain genes with phenolics.

375. Study of the bipartite promoter of the fet3-ftr1 loci of the basidiomycete *Phanerochaete chrysosporium*. Canessa, P., Larrondo, L., Agredo, M., Polanco, R. & Vicuña, R. Depto Genética Molecular y Microbiología, Fac Cs Biológicas, P Universidad Católica de Chile & Millennium Institute, Santiago, Chile.

Iron metabolism has been mainly studied in yeast. It has been shown that there are multiple systems of iron transport, which are regulated by the transcription factor Aft1. One of these is a bipartite iron transport system formed by a ferroxidase Fet3 and the permease Ftr1. Fet3 belongs to the family of multicopper oxidases, which includes laccases, ascorbate oxidases and ceruloplasmin. Ftr1 transports Fe (III) into the cell. To date, there are no studies describing an iron transport system involving Fet3 in basidiomycetes. We have recently described MCO1 in *P. chrysosporium*, a multicopper oxidase that oxidases Fe (II) to Fe (III), as it has been described for Fet3. Interestingly, MCO1 presents similarities to the Fet3 protein. To verify if MCO1 corresponds to a Fet3, we explored the *P. chrysosporium* genome. We found a homologue to the yeast *fet3* gene, just 0.8 kb away from its functional partner *ftr1*. We confirmed that *fet3* is transcriptionally active and regulated by iron. They seem to harbor a shared promoter, containing two putative boxes that match the Aft1 binding site. Using EMSA and footprinting, a probe containing the two elements mentioned above is specifically recognized by at least one nuclear protein, been both elements protected. We founded that iron has an effect on the DNA-protein complex formation.
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376. The Glyoxylate Cycle in *Penicillium marneffei*. David Cánovas, Michael J. Hynes & Alex Andrianopoulos. Dept. of Genetics, University of Melbourne, Victoria 3010, Australia

The human oportunistic pathogen *Penicillium marneffei* is a thermally dimorphic fungus. The saprofitic form is filamentous in growth at 25 ºC, resembling other *Penicillium* species. The pathogenic form is an unicellular yeast at 37 ºC, which divides by fission. To cope with nutrient deprivation during the infection process, a number of fungi employ the glyoxylate bypass. Therefore, enzymes belonging to this pathway have been implicated in pathogenesis. The glyoxylate bypass is required to replanish TCA cycle intermediates during growth on gluconeogenic carbon sources. *P. marneffei* can utilise a variety of carbon sources, including acetate and fatty acids. To gain insight into the organism's ability to utilise these carbon sources during its pathogenic process, we studied one of the key enzymes and a regulator of the glyoxylate cycle. *acuD* (isocitrate lyase) is required for growth in both acetate and fatty acids. In yeasts and *Aspergillus nidulans*, induction of *acuD* is dependent on carbon source. Surprisingly, *acuD* is mainly regulated by temperature in *P. marneffei*, being strongly induced at 37 ºC (body temperature) even in the presence of glucose and weakly induced at 25 ºC only when acetate is present. A major Zn(II)2Cys6 regulator of isocitrate lyases in other fungi was also cloned and examined in *P. marneffei*.

377. The *oxa1* Gene Controls Respiratory Complex Assembly and Lifespan in *Podospora anserina* through Interaction with the Essential *rmp1* Gene. Carole H. SELLEM, Claire LEMAIRE and Annie SAINSARD-CHANET.

A causal link between deficiency of the cytochrome respiratory pathway and lifespan was previously shown in the filamentous fungus *Podospora anserina*. To gain more insight on the relationship between mitochondrial function and lifespan, we have constructed a strain carrying a thermosensitive mutation of the gene *oxa1*. OXA1 is a membrane protein conserved from bacteria to human. The mitochondrial OXA1 protein is involved in the assembly/insertion of several respiratory complexes. We show here that *oxa1* is an essential gene in *Podospora anserina*. The $oxa1^{ts}$ mutant exhibits severe defects in the respiratory complexes I and IV correlated with an increased lifespan, a strong induction of the alternative oxidase and a reduction in ROS production. However, there is no causal link between alternative oxidase level and lifespan. We also show that in the $oxa1^{ts}$ mutant, the extent of the defects in complexes I and IV and the lifespan increase depends on the essential gene *rmp1*. The RMP1 protein, whose function is still unknown, can be localized in the mitochondria and/or the cytosolic compartment depending on the developmental stage. We propose that the RMP1 protein could be involved in the process of OXA1-dependent protein insertion.

378. Regulation of *Neurospora* Circadian Clock by an RNA Helicase Ping Cheng, Qun He, Qiyang He, Lixin Wang, Yi Liu. Department of Physiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390 USA

The eukaryotic circadian oscillators consist of autoregulatory negative-feedback loops. FRQ, WC-1 and WC-2 are three known components of the negative feedback loop of the *Neurospora* circadian clock. FRQ represses its own transcription by interacting with the WC-1/WC-2 complex and inhibiting WC's role in transcriptional activation. Here we show that all FRQ associates with FRH, an essential DEAD box-containing RNA helicase in *Neurospora*. The budding yeast homolog of FRH, Dob1p/Mtr4p, is an essential cofactor of the exosome, an important regulator of RNA metabolism in eukaryotes. Down-regulation of FRH by inducibly expression of a hairpin RNA leads to low levels of FRQ but high levels of *frq* RNA and the abolishment of circadian rhythmicities. FRH is associated with the WC complex and this interaction is maintained in a *frq* null strain. Disruption of the FRQ-FRH complex by deleting a domain in FRQ eliminates the FRQ-WC interaction, suggesting that FRH mediates the interaction between FRQ and the WC complex. These data demonstrated that FRH is an essential component in the circaidan negative-feedback loop and reveal an unexpected role of an RNA helicase in regualting gene transcription.

379. A novel transcription factor GalA, homologous to the transcriptional activator of xylanolytic and cellulolytic systems XlnR, regulates D-galactose metabolism in *Aspergillus nidulans*. Ulla Christensen, Sara Hansen, Harm Mulder, Susan Madrid and Igor Nikolaev, Danisco Innovation, Langebrogade 1, P.O. Box 17, DK 1001, Copenhagen, Denmark
A BLAST search of the *Aspergillus nidulans* Database resulted, apart from a direct homologue, in two additional hits with high similarity to XlnR, a pathway-specific transcriptional activator of xylanolytic and cellulolytic system in *Aspergilli*. Here, we present data on one of them isolated from the contig 1.75, which is mapped on chromosome III. The coding sequence of this putative transcription factor is predicted as 2.4 kb long and is interrupted by one putative intron. Like XlnR, it contains a DNA binding domain represented by a Zn binuclear cluster found proximal to its N-terminus. Juxtaposing the physical and genetic maps of *A. nidulans* suggested that the predicted regulatory gene corresponds to the *galA* locus, which has been previously characterized to control expression of galactose kinase and galactose-1-phosphate uridyltransferase, the first two steps of D-galactose metabolism in fungi. A knockout of the aforementioned regulatory gene resulted in a lack of growth on D-galactose and galactitol indicating that the encoded transcription factor, called GalA, indeed, controls the expression of genes of both the Leloir and alternative pathways of D-galactose metabolism. GalA appeared to mediate transcription of, at least, the *gal7* gene coding for galactose-1-phosphate uridyltransferase, as assessed by a Northern blot analysis. Other genes of the galactose utilization pathway were either constitutively expressed, like *gal5* (encoding phosphoglucomutase) or *gal10* (encoding UDP-galactose-4-epimerase), or only partially regulated, as *ladA* (encoding L-arabinitol-dehydrogenase required for galactitol conversion). Galactose transport apparently remained intact in the *galA* knockout, the result confirmed by a consumption rate of D-galactose similar to that of a wild type strain. A complete transcriptional profile of the genes related to D-galactose metabolism will be presented and a regulatory network will be discussed.

380. DNA Motifs in Promoters of *Ivoa, Ivob* and Other Conidiation Genes of *Aspergillus nidulans*. John Clutterbuck. Division of Molecular Genetics, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G11 6NU, Scotland, U.K.

The *ivoA* and *ivoB* genes of *A. nidulans* are required for brownish pigmentation of the conidiophore. *ivoA* encodes an N-acetyl-6-hydroxytryptophan synthase containing AMP-binding, cytochrome P450 and two condensation domains as well as a phosphopantotheine attachment site. *ivoB* encodes a copper phenol oxidase.
Both are regulated by the *brlA* gene and possibly *abaA*. Their promoters include BRE and INR the corresponding recognition sites. In order to look for other possible motifs, these and the promoters of other conidiation genes (*yA, wA, wetA, abaA, brlA, rodA and medA*) have been analysed using a series of simple Perl scripts. It is evident that promoter DNAs are far from random in sequence and contain a many distinctive motifs in addition to those already known.

381. *Neurospora crassa* Mutants with Enhanced Blue Light-dependent Gene Transcription Laura Navarro-Sampedro and Luis M. Corrochano. Departamento de Genetica, Universidad de Sevilla, Spain

The gene con-10 of Neurospora is expressed during conidiation and after illumination of vegetative mycelia. Photoactivation of con-10 is transient and disappears after two hours of light. To isolate mutants with altered gene photoactivation we have used a strain with a fusion of the con-10 promoter to the gene conferring resistance to hygromycin. This strain is sensitive to the drug when the promoter is inactive, i.e. during vegetative growth either in the dark or under continuous light. We have isolated six mutants that grow in hygromycin under continuous light but not in the dark. The mutants showed an enhanced accumulation of the con-10/hygromycin mRNA after five hours of light compared to the parental strain. The mutations in two strains only affected the photoactivation of the con-10/hygromycin fusion. Four strains showed a high and sustained photoactivation of the endogenous con-10 and, in addition, con-6. This effect was specific since the photoactivation of genes al-2, vvd, and wc-1 was normal in the mutants. The threshold for con-10 and con-6 photoactivation was not altered suggesting that the phenotype is due to a higher transcriptional rate or mRNA stability after a light stimulus. Mutations in genes vvd, encoding a flavoprotein, rco-1, encoding a putative gene repressor, and rco-3, encoding a putative glucose sensor, also resulted in enhanced and sustained photoactivation of con-10 and con-6. Our results support a complex mechanism composed at least of VVD, RCO-1, and RCO-3 responsible for transient gene photoactivation.

382. The Gene for the Heat-shock Protein Hsp100 Is Induced by Blue Light and Heat-shock in *Phycomyces blakesleeanus*. Julio L. Rodríguez-Romero and Luis M. Corrochano, Departamento de Genética, Universidad de Sevilla, Spain

The Phycomyces hspA gene product is a 901 amino acid-protein member of the clpB/HSP100 family. HSP100 proteins are ATPases involved in the tolerance to high temperatures, proteolysis, and protein disaggregation. The hspA promoter contains three heat-shock elements that are presumably involved in the activation of hspA after a heat-shock. In addition, four sequences are present in the hspA promoter and in the promoter of the photoinducible genes carB and carRA, and are candidates for binding sites for light-regulated transcription factors. Blue light can activate transcription of the hspA gene by 10-fold with a threshold of 1 J/m^2. The threshold for hspA photoactivation is 10^4 times higher than the thresholds for blue-light regulation of sporangiophore development and photocarotenogenesis, which suggests that there are differences in the photosensory systems for gene photoactivation and mycelial photoresponses. A heat-shock of 30 min at 34ºC or 42ºC activated the hspA gene by 160-fold. The differences in maximum hspA gene transcription by blue light and heat shock suggest the presence of different regulatory mechanisms.
Mutations in genes madA and madB impair all photoresponses, including the photoactivation of hspA but did not alter hspA activation by heat-shock. Mutations in car genes required for beta-carotene biosynthesis reduced or impaired hspA photoactivation. Our results suggest that mad and car gene products are required for hspA photoactivation in a complex photosensory system.

383. The Neurospora Circadian Clock is Composed of Multiple Oscillators. Renato Magalhaes de Paula, Zachary Lewis, Kyung Suk Seo, Andrew Greene and Deborah Bell-Pedersen*. Department of Biology, Texas A&M University, College Station, TX 77843

Circadian clocks coordinate daily changes in behavior, physiology, and gene expression in organisms. In Neurospora crassa, the clock regulates the production of conidia and the rhythmic expression of a number of genes and proteins. Using microarrays, we identified an evening-specific gene, ccg-16, that has rhythms in mRNA accumulation that persist in the absence of FRQ in constant darkness and in constant light. These data establish that ccg-16 is controlled by a novel oscillator that does not require FRQ. We call this oscillator the UFO for un-FRQ oscillator that regulates an evening-specific gene. The UFO appears to be coupled to the FRQ-based oscillator through the products of the wc-1 and wc-2 genes. Null mutations in either wc-1 or wc-2 abolish ccg-16 rhythms, and the phase of the ccg-16 rhythm is altered in a long-period frq7 mutant allele in cultures grown in constant darkness. Together, these data demonstrate that the circadian system of Neurospora involves multiple-coupled oscillators that contribute to the diversity and refinement of the output pathways.

384. Regulation of the pentose catabolic pathway genes of *Aspergillus niger*. M.J.L. de Groot[1], L. Vandeputte-Rutten,[2], C. van den Dool[3], H.A.B. Wösten[3], M. Levisson[1], P.A. vanKuyk[1], G.J.G. Ruijter[1], and R.P. de Vries[3]. [1]Fungal Genomics, Wageningen University, The Netherlands; [2]Department of Crystal and Structural Chemistry, Utrecht University, The Netherlands; [3]Microbiology, Utrecht University, The Netherlands.

The aim of this study was to obtain a better understanding of the pentose catabolism in *Aspergillus niger* and the regulatory systems that affect it. To this end, we have cloned and characterised the genes encoding the *A. niger* L-arabitol dehydrogenase (*ladA*) and xylitol dehydrogenase (*xdhA*), and produced the enzymes in *Escherichia coli* to determine the substrate specificity.
Expression analysis of the pentose catabolic pathway genes resulted in a model in which an L-arabinose specific regulator activates the expression of all genes required for the conversion of L-arabinose to D-xylulose-5-phosphate. In addition, XlnR regulates the first step and, to a lesser extent, the other steps of the conversion of D-xylose into D-xylulose-5-phosphate. This model explains all reported data for the expression of pentose catabolic genes, arabinolytic genes and xylanolytic genes. It also takes into account the overlapping substrate specificities of the pentose catabolic enzymes that effect the accumulation of L-arabitol, the inducer of the L-arabinose catabolic pathway genes and the arabinolytic genes.

385. Hog Map Kinase Controls Stress Response in T. harzianum. Delgado-Jarana, J.[1], Sousa, S.[1], Monte, E., Rey, M.[2], Llobell, A[1]. [1]Instituto de Bioquímica Vegetal y Fotosíntesis. Universidad de Sevilla. Av. Americo Vespucio s/n. 41092 Sevilla. Spain. [2]Newbiotechnic, S.A. Parque Industrial de Bollullos A-49 (PIBO). 41110 Bollullos de la Mitación. Sevilla. Spain.

Trichoderma harzianum is a soil-borne fungus, able to colonize the rhizosphere displaying antagonistic activities against phytopathogenic fungi and developing mycorrhiza-like interactions with plant roots. In the soil, *T. harzianum* is exposed to a highly variable environment with respect to the availability of nutrients, temperature, pH, oxygen and water. All these changes have to be sensed by the fungal cell to adapt its metabolism and to develop the appropriate response.
We have characterized the *T. harzianum hog1* gene, which encodes a MAPK involved in stress response. Hog1 is phosphorylated under oxidative stress, hyperosmotic shock, presence of heavy metals and other conditions. We have analysed hyperactive mutants (carrying F314S mutation) and a silenced strain (using a hairpin construct) with respect to stress resistance and cross protection, showing the key role of Hog1 in stress response. *hog1* was able to complement *hog1delta* mutation in yeast, but not *hog1delta pbs2delta* which may be related to different sequence features and/or functional divergent role of Hog1 protein in both systems. On the other hand, we have carried out arrays hybridization using to study hyperosmotic shock transcription profile. From these data, a clear view of the genes induced under stress, and the potential co-regulation in plant-fungus association can be established.

386. Mutations in the gene encoding the mitochondrial DNA polymerase affect differentially the *Podospora anserina* lifespan in different genetic contexts. Michelle Dequard-Chablat, Veronique Contamine, Marguerite Picard, and Robert Debuchy. Institut de Genetique et Microbiologie, UMR8621, Orsay, France.

Podospora anserina has a limited lifespan, which is measured as the length of mycelium from ascospore germination. In our laboratory culture conditions, a wild-type strain has a lifespan of 10 cm and displays alterations of the mitochondrial genome at the time of death. A mutation in the *AS1* gene, encoding a cytosolic ribosomal protein, reduces the lifespan to 1.4 cm and leads to the accumulation of a specific deletion of the mitochondrial genome at the time of death. A screen for suppressors increasing the lifespan of the *AS1* mutant strain led to the isolation of 4 different mutations in the gene encoding the mitochondrial DNA polymerase (polG). All these mutations map in the C-ter part of the polG, an evolutionnary non-conserved region of unknown function. In an *AS1* mutant strain, these suppressors increase the lifespan to a value higher than the mean longevity of a wild-type strain, while in an $AS1^{+}$ strain, the *polG* suppressors decrease the lifespan to a value below the lifespan of a wild-type strain. We are currently performing a physiological and genetic analysis of these mutants and of other site-directed mutations in evolutionnary conserved positions of the polG. The genetic interactions of these mutations with previously obtained suppressor mutations mapping outside the *polG* gene open the possibility to identify the polG partners.

387. Gene Regulation During Nitrogen Stress and Invasive Plant Growth in the Rice Blast Pathosystem. Nicole Donofrio and Ralph A Dean. NCSU, 851 Main Campus Drive, Raleigh, NC

Previous research on *Magnaporthe grisea*, causal agent of the rice blast disease, suggests that during certain stages of its invasive growth cycle, the pathogen is in a nitrogen-limited environment. To gain insight into *M. grisea* physiology during nutrient stress and invasive growth, we examined global gene expression changes under nitrogen starvation conditions in culture. Using the whole genome, oligo microarrays were designed in collaboration with Agilent Technologies, and contain 13,666 *M. grisea* genes. Approximately nineteen hundred genes showed significantly increased expression during nitrogen starvation. Among these, we found an increase in expression level of nitrogen scavenging genes, such as the global nitrogen regulator in *M. grisea*, *NUT1*. Furthermore, we found several genes with known roles in pathogenicity, including *PTH11* and *MPG1*, to be highly expressed during nitrogen starvation after both 12 and 48 hours. We confirmed the expression patterns of 20 up-regulated genes using reverse transcription PCR, providing a confirmation of our microarray results. To determine whether nitrogen regulatory genes were also expressed during infection, we used RT-PCR to examine expression patterns of several genes during invasive growth of *M. grisea* in rice. These data will be presented, along with preliminary data from targeted deletion lines of several genes differentially regulated during nitrogen stress.

388. Nitrogen metabolite represion of arginine catabolism genes in *Aspergillus nidulans* is mediated by negatively acting factor AREB. Agnieszka Dzikowska[1] , Piotr Weglenski[1] and Claudio Scazzocchio[2] . [1]Department of Genetics Warsaw University, ul. Pawinskiego 5a 02-106 Warsaw, Poland; [2]Institut de Génétique et Microbiologie, Université Paris-Sud, UMR 8621, Bat. 409, 91405 Orsay CEDEX, France

The arginine catabolism genes *agaA* and *otaA* , coding for arginase and ornithine transaminase (OTAse) respectively, are specifically induced by arginine and repressed by ammonia. In *A. nidulans* nitrogen metabolite repression is mediated by transcriptional activator AREA from GATA family. *areA 600* loss of function mutant does not grow on arginine as a nitrogen source but we have shown that *agaA* and *otaA* expression does not depend on AREA. Arginase and OTAse enzymatic activities are fully inducible in *areA600* mutant. The same was shown for mRNAs. It is possible that an arginine permease gene(s) can be a target for AREA activator. Preliminary data suggest that there are two arginine transport systems and only one depends on AREA activator. We have also shown that another negatively acting factor from GATA family (AREB) participates in the ammonia repression of *agaA* and *otaA* . In *areB* loss of function mutant the ammonia repression of both genes is changed at the level of enzyme activity and at the level of mRNA.

389. Characterization of a sugar sensing pathway in *Neurospora crassa*. Xin Xie, Heather H. Wilkinson, and Daniel J. Ebbole. Program for the Biology of Filamentous Fungi, Dept. of Plant Pathology and Microbiology Texas A&M University, College Station, TX

Approximately 19% of 1385 *Neurospora crassa* genes represented on a cDNA microarray are regulated following a shift from medium containing glucose to carbon starvation medium. Glucose transport activity and expression of a high-affinity glucose transporter gene is induced in response to this shift. We previously hypothesized that one mechanism for sensing glucose involves the use of glucose transporter homologs as glucose receptors. The *rco-3* gene is a regulator of sugar transport and conidiation in *N. crassa* and we isolated suppressors that remedy the sorbose resistance phenotype of the *rco-3* mutant. A previously characterized mutant defective in the *dgr-1* gene phenotypically resembles *rco-3*. To test whether *dgr-1* is involved in the *rco-3* signaling pathway, epistasis relationships among *rco-3, dgr-1* and the suppressors were examined. To further investigate the relationship between *rco-3* and *dgr-1* and to help assess the extent to which they may constitute a common regulatory cascade, the transcriptional response to glucose status in *rco-3* and *dgr-1* mutants was examined via microarrays. *rco-3* and *dgr-1* are both required for appropriate regulation of genes involved in the TCA cycle, the glyoxylate cycle and gluconeogenesis. We conclude that *rco-3* and *dgr-1* form part of a common pathway to regulate the cellular response to carbon source.

390. Cyclic-AMP dependent kinases facilitate inactivation of nitrogen regulation by newly formed Ure2p amyloid in S. cerevisiae. Herman K. Edskes, Benedetta M. Naglieri, Reed B. Wickner. Laboratory of Biochemistry and Genetics, NIDDK, NIH

The Saccharomyces cerervisiae Ure2 protein regulates the transcription factor Gln3p that targets genes needed for the uptake and utilization of poor nitrogen sources. Thus in the presence of a good nitrogen source like ammonium the Dal5 permease, capable of taking up the poor nitrogen source allantoate, is not expressed. However, in approximately one in a million cells the nitrogen regulation system (NCR) is inactivated due to Ure2p aggregating as infectious (prion) amyloid filaments. Overexpression of Ure2p greatly enhances initiation of amyloid formation. Deletion of MKS1, a negative regulator of the Rtg1p and Rtg3p transcription factors that direct transcription of glyoxylate and tricarboxylic acid cycle genes, prevents activation Dal5p by newly seeded Ure2p amyloid but not by established Ure2p filaments. Deletion of RTG3 is epistatic to deletion of MKS1. However, glutamate, a potent repressor of RTG1/3 directed transcription does not affect Dal5p activity in a MKS1 deletion strain. Deletion of both TPK1 and TPK3, two cyclic-AMP dependent kinases, also prevents activation of Dal5p by newly seeded Ure2p amyloid. Again this effect is overcome when RTG3 is deleted.

391. VVD's role in entrainment of the *Neurospora crassa* circadian clock. Mark Elvin & Christian Heintzen. Faculty of Life Sciences, The Michael Smith Building, The University of Manchester, Manchester, M13 9PT, UK

To provide an accurate depiction of external time, *Neurospora's* circadian clock is synchronized to the rhythmic environment via a process called entrainment. Light and temperature are important entrainment cues. We previously identified VVD as a small PAS/LOV protein that is clock-controlled, rapidly light induced and, although not essential for circadian rhythmicity, influences light sensitivity and the phase of the circadian clock. Our recent work has focused on elucidating VVD's significance for the circadian clockwork by investigating the molecular and phenotypic differences between *vvd* knockout and wild-type strains in various light-dark entrainment conditions. Data will be presented that illuminate the role of VVD in entrainment of the *Neurospora* circadian clock to light-dark cycles and we propose a molecular mechanism by which VVD facilitates light-dark entrainment.

392. Inactivation of the histidine-kinase *Bcos1* of *Botrytis cinerea* has pleiotropic effects on fungal growth, development, and virulence. Sabine Fillinger[1], Weiwei Liu[1], Laurent Legendre[2], Pierre Leroux[1], Jai Santosh Polepalli[2], and Muriel Viaud[3]. [1]UPMC, INRA Versailles, Route de Saint-Cyr, 78026 Versailles, France. [2]Centre for Horticulture and Plant Sciences, University of Western Sydney, Locked bag 1797, Penrith South DC, NSW 1797, Australia, [3] PMDV, INRA Versailles, Route de Saint-Cyr, 78026 Versailles, France

Filamentous ascomycetes contain large numbers of histidine-kinases (HK) most of which are highly conserved among species (Catlett *et al*, 2003; *Eukaryot. Cell* 2, 1151)). We have inactivated the HK of group III, *Bcos-1*, in the economically important plant pathogen *Botrytis cinerea*. The *Bcos-1* gene was previously involved in fungicide resistance (Cui *et al*., 2002; *FGB* 36, 187). A detailed phenotypic analysis shows that Bcos-1 deletion has pleiotropic effects on the fungus. Besides the expected fungicide resistance and osmosensitivity also the asexual development is altered (reduced growth, no conidiation). However, the mutants do not present higher sensitivities to other stress-conditions, but are more resistant to menadione (vitamine K3) provoking oxidative stress. Osmosensitivity and reduced protoplast-formation moreover suggest alterations in cell-wall composition. Pathogenicity tests show that Bcos-1 mutants are severely impaired for plant infection. The BcOs1 histidine kinase therefore constitutes a major pathogenicity factor of *B. cinerea*. Presumably it regulates a signal-transduction cascade involved in osmosensing comparable to the HOG (high-osmolarity glycerol) pathway of *Saccharomyces cerevisiae*. In contrast to the yeast Sln1 sensor histidine kinase, BcOs1 does not have a detectable transmembrane domain. Using a BcOs1-GFP fusion we can show the fluorescence localised in the cytoplasm. The cytoplasmic localisation of BcOS1 raises the question if the histidine-kinase is the sensor protein of external osmotic pressure or if this function is taken over by an additional sensor protein. Transcriptome analyses on 3032 unigenes are being carried out under various conditions between the deletion mutants and the parental strain in order to combine gene-expression data and phenotypes.

393. Regulation of the biosynthesis of the toxin, sirodesmin, in the ascomycete *Leptosphaeria maculans*. Ellen M. Fox, Donald M. Gardiner and Barbara J. Howlett. University of Melbourne, Melbourne, VIC, Australia

Sirodesmin PL is a phytotoxin produced by the fungus *Leptosphaeria maculans*, which causes blackleg disease of canola (*Brassica napus*). A cluster of 18 genes with predicted roles in the biosynthesis of sirodesmin PL has been cloned. Attempts are being made to determine the roles of individual genes in the cluster via RNA interference, as well as by targeted mutagenesis. The knock down of *sirZ*, a putative transcription regulator of the cluster, resulted in a dramatic reduction in sirodesmin production. Additionally genes elsewhere in the genome that regulate sirodesmin biosynthesis are being sought. An assay, which exploits the antibacterial properties of sirodesmin, has been developed to screen for sirodesmin deficient mutants. This screen has already yielded five mutants, which are currently being analysed.

394. *In vitro* sporulation of the obligate plant pathogen *Ustilago maydis*. Maria D. Garcia-Pedrajas and Scott E. Gold. Department of Plant Pathology, University of Georgia, Athens, GA

The repression of a subset of genes normally expressed in budding cells, upon switch to filamentous growth may be critical for programming morphogenesis and virulence in the corn pathogen *Ustilago maydis*. To identify these filament downregulated genes and to better understand the role of wild-type budding cells in the life and disease cycle of *U. maydis* in nature, we used suppression subtractive hybridization (SSH). We screened 5,500 cDNA clones by an iterative sequencing and hybridization process. Greater than 88% of the differentially expressed clones detected corresponded to 48 different genes. Differential expression (*2 fold) was confirmed for 37 of these genes by northern blot analysis. We have produced deletion mutants in several of the highly differentially expressed genes. The effect of these mutations on morphology and/or pathogenicity will be discussed. Additionally, analysis of the putative promoter region of these filament downregulated genes identified a potential regulatory sequence, which led us to the isolation of a new regulator (*ust1*) of morphogenetic processes in *U. maydis*. Deletion of *ust1* in wild-type budding cells and in solopathogenic haploid and diploid backgrounds resulted in filamentous growth, with areas devoid of cytoplasm. Additionally, highly melanized thick-walled structures, closely resembling teliospores produced in plant tumors, were produced *in vitro* in these mutants. We are currently in the process of fully characterizing this newly identified transcription factor and its role as a repressor of sporulation.

395. Expression of *Fost20*, a PAK Kinase Gene from the Phytopathogenic Fungus *Fusarium oxysporum*. M. A. García-Sánchez, B. Ramos, N. Martín-Rodrígues, A. P. Eslava and J. M. Díaz-Mínguez. Area de Genética, Centro Hispano-Luso de Investigaciones Agrarias (CIALE) Universidad de Salamanca 37007, Salamanca, Spain

Fusarium oxysporum is a soil-borne plant pathogen that causes wilt disease in a variety of crops. The fungus enters the plant through the roots and to spread throughout entire vascular system, causing characteristic wilt symptoms. To perform these processes the fungus must perceive chemical and physical signals from the host plant and respond with the appropriate metabolic and morphological changes required for disease development. Many of these responses have been shown to be dependent on conserved signal transduction pathways.
In eukariotic cells, a family of serine/threonine protein kinases (PAK kinases) are involved in various cellular signaling and developmental processes. To understand the role of PAK kinases in activating the MAP kinase pathway, we isolated and characterized the Ste20 homologous in *F.oxysporum*, named *fost20*. The *fost20* mutants were reduced in conidiation but had no obvious defect in pathogenicity and/or virulence. Although it is not clear how nutritional conditions affect these genes, one possibility is that the MAP kinase pathway involved in nutrient or pH sensing may be affected in *fost20* mutants. Thus, we have analysed whether there is any differential expression of these genes under nutrient starvation conditions, and pH stress using Real-Time PCR and Norhern analysis.

396. Culture-dependent alternative transcription initiation of the enolase-encoding gene (*enoA*) in *Aspergillus oryzae*. Katsuya Gomi[1], Yuuichi Yamaki[1], Takeshi Akao[2], Tomomi Toda[3], Masayuki Machida[3]. [1]Graduate School of Agricultural Science, Tohoku University, Sendai, [2]National Research Institute of Brewing, Higashi-Hiroshima, [3]National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan.

Enolase generally plays a pivotal role in the glycolysis and is therefore one of the most highly expressed proteins in most organisms. We have cloned an enolase-encoding gene (*enoA*) of *Aspergillus oryzae* and identified a *cis*-element of 15-bp located between -195 and -181 relative to the translation start codon responsible for the transcriptional regulation of the *enoA* by promoter deletion analysis and gel mobility shift assay[1)]. Surprisingly, recent comparative analysis using ESTs and whole genomic sequence data of *A. oryzae* suggested the existence of alternative transcription initiation sites in the *enoA* dependent on culture conditions. In the absence of carbon source or in carbon-limited cultures, transcription of the *enoA* is initiated mainly at -530 upstream of the start codon and results in the formation of transcript with an intron of approximately 440-bp in the 5• f-untranslated region (5• fUTR) removed. In contrast, the *enoA* is transcribed from -50 relative to the start codon, which is located within the intronic sequence in the 5• fUTR, in the presence of carbon sources such as glucose. To our knowledge, this is the first finding that alternative transcription initiation sites are utilized depending on culture conditions in the genes involved in the primary metabolic pathway in filamentous fungi, except for the developmental regulatory gene, *brlA*, of *A. nidulans*[2)].
[1)] T. Toda et al., *Curr. Genet.*, **40**, 260-267 (2001). [2)]R. Prade and W. Timberlake, *EMBO J.* , **12**, 2439-2347 (1993).

397. Both carbon and nitrogen regulate expression of genes involved in nitrogen translocation between partners in the arbuscular mycorrhizal symbiosis. Manjula Govindarajulu[1], Philip E. Pfeffer[2], Yair Shachar-Hill[3], Peter J. Lammers[1]. [1]Dept. Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA. [2]USDA-ARS Eastern Regional Research Ctr., 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA. [3]Plant Biology Dept., Michigan State University, East Lansing, MI 48824-1312, USA.

Arbuscular mycorrhizal fungi (AMF) supply nutrients including nitrogen to host plants in return for carbon derived from photosynthesis. Analysis of N and C flux using stable isotopes supports a model whereby AMF shuttle nitrogen to their host plants via assimilation of inorganic N into arginine and translocation of this amino acid to fungal tissue within the host plant where the catabolic arm of the urea cycle releases N as ammonia to the host plant with no loss of fungal C. We have measured fungal mRNA levels for key N enzymes and transporters in both extraradical (ERM) and intraradical mycelium (IRM) tissues using quantitative real-time PCR. Glutamine synthase (GS) was shown to be expressed in both ERM and IRM tissues isolated from the carrot hairy root co-culture system. GS transcripts were significantly higher when NH4+ was substituted for NO3- in the ERM compartment of an in vitro split plate culture system. Consistent with predictions from the model, the same conditions lead to significantly higher mRNA levels in the IRM tissues for two urea cycle enzymes [urease accessory protein (UAP), ornithine aminotransferase (OAT)] and an ammonium transporter (AMT). Conversely, expression of the fungal AMT, OAT and UAP genes was down-regulated in IRM tissues as glucose levels in the medium dropped. This was reversed by supplemental glucose demonstrating that regulation of key fungal N genes is dependent on carbon availability. The same pattern of down regulation of fungal AMT, OAT and AMT was observed when pot-grown Medicago sativa plants colonized by Glomus intraradices were placed in the dark to limit carbon availability. These results show that nutrient exchange in the symbiosis is tightly controlled and suggests a mechanism for regulation of coupled nutrient exchanges between the symbionts.

398. Nonsense-mediated mRNA decay in yeast regulates global gene expression through a network of direct and indirect targets. Qiaoning Guan and Michael R. Culbertson. Laboratories of Genetics and Molecular Biology, University of Wisconsin, Madison, WI 53706

Nonsense-mediated decay (NMD) is a eukaryotic mechanism that rapidly degrades messenger RNAs containing premature termination codons (PTC). Recently, several genome-wide expression profiles in yeast and mammals indicate that NMD may serve a post-transcriptional regulatory role in controlling the steady-state levels of several hundred wild-type mRNAs. Similar physiological categories of the natural substrates of NMD have been identified in the yeast *Saccharomyces cerevisiae* and humans. Although several molecular mechanisms have been proposed for how NMD targets specific mRNAs for altered decay, many of the mRNAs whose abundance is influenced by NMD do not show altered decay rates. We are investigating a model of direct and indirect targeting by the NMD pathway that includes transcription factors and the genes they regulate, resulting in a complex network of changes in mRNA abundance. mRNAs coding for transcriptional activators and repressors are candidates for direct NMD targets, although some of these can be indirect targets if they function in a regulatory network of multiple transcription factors. The direct target mRNAs exhibit a change in half-life when NMD is inactivated. Other genes whose expression is regulated by a targeted transcription factor become substrates of NMD as an indirect effect. Indirect targets exhibit a change in mRNA abundance due to the change in rate of transcription. We have determined the decay kinetics of a list of mRNAs coding for transcription factors in isogenic NMD^+ and nmd^- strains, and have identified both direct and indirect targets. The transcription factor *FZF1* mRNA contains an upstream open reading frame (uORF) which overlaps with its protein-coding region. Eliminating the uORF by mutating its start codon from AUG to AGG results in diminished sensitivity of the *FZF1* mRNA to NMD, suggesting that the uORF plays a role in but is not completely responsible for the targeting of the *FZF1* mRNA. To fully understand how the mRNA abundance including *FZF1* and other transcription factors is directly affected by NMD, we are using visual analysis of meiotic tetrads expressing *GFP* fusions to delineate the destabilization elements of these mRNAs. Further discrimination between direct and indirect targets of NMD coupled with an analysis of transcriptional regulatory circuits will allow us to generate a blue print of the global gene expression regulation in the subset of yeast genes whose expression is influenced by the NMD pathway.

399. Differential gene expression during perithecial development in *Gibberella zeae* (anamorph *Fusarium graminearum*). Heather E Hallen, Weihong Qi, Frances Trail. Michigan State University, Department of Plant Biology, East Lansing, MI 48824

Gibberella zeae (anamorph *Fusarium graminearum*) is the causal agent of Fusarium head blight, a disease of wheat, corn and other crop plants causing an estimated three billion dollar loss in the US alone during the 1990's. The fungus overwinters on crop debris and ascospores are discharged in the spring to infect the new crop. As sexual development and spore discharge are thus crucial features of the infection cycle, we are investigating the genes involved in different stages of sexual development. We designed and used a cDNA microarray representing 2067 genes to investigate differential expression. Currently, we are conducting genome-wide studies using Affymetrix gene chips representing all predicted genes, ORFs and ESTs of *Fusarium graminearum*. Knockouts on genes likely to be involved in sexual development and ascospore discharge are underway.

400. Investigating abnormal phenotypes associated with RNA silencing enzymes and RNA-dependent RNA polymerases in *Aspergillus nidulans*. T. M. Hammond[1], Y. Reyes Domínguez[2], L. R. Milde[1], J.W. Bok[1], C. Scazzocchio[2] and N. P. Keller[1]. [1]Plant Pathology, University of Wisconsin, Madison, WI. [2]Institut de Génétique et Microbiologie, Université Paris-Sud, France.

Of the three Aspergilli with fully sequenced and publicly available genomes, *A. fumigatus*, *A. nidulans*, and *A. oryzae*, *A. nidulans* encodes the fewest enzymes commonly attributed to RNA silencing related processes. For example, *A. nidulans* is predicted to have only one ‘Dicer', one Paz and Piwi domain (PPD) protein and two RNA dependent RNA polymerases (RDRPs), while the other two sequenced Aspergilli, and most other studied filamentous ascomycetes, are predicted to have at least two Dicers, two PPD proteins, and three RDRPs. The reduced number of these types of genes in *A. nidulans* suggests that it is an ideal model organism to study the biological function of these genes in filamentous fungi. In previous studies we have shown that the predicted *A. nidulans* PPD protein (RsdA) is required for RNA silencing during the vegetative cycle but that its two RDRPs (RrpA and RrpB) are not required for the same process. Interestingly, the simple experiments required to determine that the one predicted Dicer (DcrA) is required for RNA silencing in *A. nidulans* have not yet been completed because of an abnormal level of difficulty encountered during our attempts to obtain a *dcrA* deletion strain. In addition to our continual pursuit of a *dcrA* deletion strain, we are currently investigating two peculiar phenotypes associated with RNA silencing related genes but not thought to be directly related to RNA silencing during the vegetative cycle. These include a ‘slow-growth' phenotype associated with *dcrA* over-expression and a ‘selfing proficient' but ‘crossing deficient' phenotype associated with *rrpB* deletion.

401.Withdrawn

402. Galactokinase activity is essential for high cellulase transcript levels during growth on lactose in *Hypocrea jecorina*. L. Hartl, B. Seiboth, C.P. Kubicek. Institute of Chemical Engineering, TU Vienna

H. jecorina (*Trichoderma reesei*) is a producer of important extracellular enzymes, e.g. cellulases and hemicellulases. The *H. jecorina* galactokinase encoded by *gal1* catalyses the first step of the D-galactose degrading Leloir pathway. Strains deleted in *gal1* show reduced growth on D-galactose and reduced cellulase transcript levels while growing on lactose.
In *Kluyveromyces lactis* galactokinase is bifunctional: it catalyses D-galactose phosphorylation, and also signals D-galactose-induction to the *GAL* genes via interaction with Gal80p.
To define the role of the *H. jecorina* galactokinase in cellulase induction the following strains were constructed by transforming a *gal1* deleted strain: Strains expressing (i) an enzymatically inactive galactokinase by deletion of two essential amino acids (Gal1-SA), (ii) a solely enzymatically active galactokinase by using the *galK* from *E. coli* and (iii) the *gal1* gene under the *pki1* promoter. Only strains expressing *galK* or the *H. jecorina* galactokinase under the *pki1* promotor showed increased growth rates on D-galactose indicating that the Gal1-SA had lost its galactokinase activity. Cellulase induction by lactose was dependent on galactokinase activity since induction was found in strains expressing the *E. coli* galactokinase or the *H. jecorina* galactokinase under the *pki1* promotor but not in the Gal1-SA expressing mutant.

403. Molecular cloning and characterization of two hydrophobins and a Cerato ulmin-like protein from the entomopathogenic fungus *Beauveria bassiana*. Diane Holder, Eun-Min Cho, and Nemat O. Keyhani. University of Florida, Microbiology and Cell Science, Bldg 981, Museum Rd. Gainesville, FL 32611

Hydrophobins are small predominantly hydrophobic, amphipathic proteins that function in a broad range of growth and developmental processes in fungi. They are involved in the formation of aerial structures, the attachment of fungal cells to surfaces, and act in signaling in response to surface cues and pathogenesis. *Beauveria bassiana* is an important entomopathgenic fungus used as an arthropod biocontrol agent. To examine the feasibility of using phage display technology to clone cDNAs encoding hydrophobins, we performed biopanning experiments using a variety of affinity resins, including N,N'-diacetylchitobiose, fucose, lactose, maltose, and melibiose-coupled agarose beads. After 5 rounds of iterative biopanning, cDNAs corresponding to three *B. bassiana* hydrophobins were isolated, one of which displayed high homology to the toxin, Cerato-ulmin. Suppressive subtractive hybridization (SSH), used to isolate transcripts induced during fungal growth on chitin, also resulted in the isolation of one of the *B. bassiana* hydrophobins. These results reveal the differential regulation of the isolated hydrophobins and indicate that phage display may represent a novel approach to cDNA cloning of hydrophobins.
This work was support it part by NSF grant # EF-0412137 (NOK).

404. Antisense *frequency* (*qrf*) RNA and its role in the circadian clock of *Neurospora crassa*. Suzanne Hunt, Zoulikha Mohammed, Matthew Mayho, Marion Hogg & Susan Crosthwaite. Faculty of Life Sciences, The Michael Smith Building, The University of Manchester, Manchester, M13 9PT, UK

Rhythmic levels of *frequency* (*frq*) messenger RNA are essential for maintaining circadian rhythmicity in the filamentous fungus *Neurospora crassa*. Apart from mRNA transcripts encoding the FRQ protein, antisense *frq* (*qrf*) transcripts are also transcribed and these have been shown to affect the response of the clock to light (Kramer *et al*., 2003 Nature 21:948-952). Altering the expression of *qrf* does not affect the clocks ability to entrain to temperature cycles. However, preliminary data indicate that when temperature pulses are administered at different times of the circadian day the response of *qrf*-defective strains differs from the wild type. *frq* and *qrf* transcripts are *cis*-encoded and complementary over large regions. Therefore, we investigated whether or not their levels might be regulated via the formation of double-stranded RNA and present data on the circadian properties of quelling-defective mutants.

405. Identification of a Natural Antisense Transcript of *aflR*, the Transcriptional Regulator in the Aflatoxin Biosynthesis Pathway in *Aspergillus flavus*. Carrie Jacobus[1], Gary Payne[2], and Niki Robertson[1,3]. [1]Department of Genetics. [2]Department of Plant Pathology. [3]Department of Botany. NCSU Raleigh, North Carolina.

Natural antisense transcripts (NATs) are involved in gene expression in several eukaryotic systems. Cases of transcriptional interference, RNA masking, and double-stranded RNA dependent mechanisms have all been shown to involve NATs. Recently, antisense transcripts have been identified in the filamentous fungus *Neurospora crassa* that are complementary to *frq*, a key component of the circadian clock. An antisense transcript was identified in a cDNA library of another filamentous fungus, *Aspergillus flavus*. This species among others in the genus *Aspergillus* produces aflatoxin, the most potent naturally occurring carcinogen. The antisense transcript identified in the cDNA library corresponds to *aflR*, which is the transcriptional regulator of aflatoxin biosynthesis. This NAT, *aflRas*, overlaps with the 5' end of *aflR* and extends into its promoter. RT-qPCR analysis was used to compare *aflR* and *aflRas* levels with respect to aflatoxin production at different time points. In addition, an over-expression construct utilizing the *alcA* promoter to drive expression of *alfRas* was transformed into *A. flavus*. These over-expression transformants will provide insight as to the function of *aflRas*. **(Presented at poster number 538)**

406. Characterization of 14-3-3 homolog in the rice blast fungus, *Magnaporthe grisea*. Jun Seop Jeong and Ralph A. Dean. North Carolina State University, CIFR(Center For Integrated Fungal Research).

14-3-3 protein is a small acidic protein that is found universally in eukaryotes. In contrast to animals and plants, only one or two homologs are usually found in fungi. Despite the extensive researches in yeast species, little is known in regard to its role in filamentous fungi. The protein is implicated in signal transduction, cell cycle, nutrient utilization, vesicular trafficking, growth, and development. However, due to the considerable sequence divergence and functional overlap of the gene family, the precise role 14-3-3 proteins play in cellular processes remains obscure. In the rice blast fungus, one 14-3-3 homolog is annotated (MgFTT1) in the current *Magnaporthe grisea* genome database (http://www.broad.mit.edu/ annotation/fungi/magnaporthe/). Further analysis revealed the presence of, at least, one more homolog in the genome. To investigate the function of MgFTT1, gene knockout mutants were created. The phenotypic consequences of targeted deletion will be presented.

407. Global patterns of gene regulation associated with the development of ectomycorrhiza between birch and *Paxillus involutus*. Tomas Johansson, Antoine Le Quéré, Derek Wright, and Anders Tunlid. Microbial Ecology, Lund University, Sweden

The formation of ectomycorrhizal (ECM) root tissue is characterized by distinct developmental stages including pre-infection/adhesion, mantle and Hartig net formation. The global pattern of gene expression along the development of an ECM association between birch (*Betula pendula*) and *Paxillus involutus* was analysed using cDNA microarrays. In comparison to non-symbiotic tissue, 251 fungal genes (of 1,075 in total) and 138 plant genes (of 1,074 in total) were differentially regulated during the ECM tissue development and clusters of coregulated plant or fungal were identified. For example, during mantle and Hartig net development, an upregulation of plant genes known to be involved in plant defense responses elicited during fungal pathogen challenge occurred. Notably, this response was significantly reduced in fully developed ECM tissue. Among fungal genes several homologues encoding proteins involved in the respiratory chain were upregulated during mantle and Hartig net formation. However, the relative expression level of these genes decreased during later stages of ECM tissue development. Within fully developed ECM tissue, there was significant upregulation of fungal genes related to protein synthesis and components of the cytoskeleton assembly machinery. This study highlights the complex molecular interactions which occur between symbionts during the development of ECM associations.

408. Analysis of Two-component Histidine Kinases, and Several Downstream Effectors in the Filamentous Fungus, *Neurospora crassa*. Carol A. Jones, Suzanne Phillips, and Katherine A. Borkovich Department of Plant Pathology, University of California, Riverside, CA, USA 92521

Two-component systems consist of proteins that contain a histidine kinase and/or a response regulator domain. Histidine kinases (HKs) signal via a phosphorelay. In complex two-component signaling, the HK is of the hybrid type, meaning that the HK and the RR domains are within the same protein. The HK is autophosphorylated in response to an environmental signal; this phosphate is then transferred to the response regulator (RR) domain, which then gets transferred to a histidine phosphotransferase (HPT), and then onto a second RR. In various organisms the HK pathway helps regulate environmental responses to light, nutrient and oxygen levels, osmolarity, and other factors. In eukaryotes, HKs are involved in the activation of mitogen-activated protein kinase (MAPK) cascades and/or transcriptional regulation.

The completion of *Neurospora crassa* genome has revealed eleven putative HKs, one HPT, and two RR genes. The number of HKs found in *Neurospora* is considerably larger than that of the sequenced yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. I am in the process of producing *Neurospora* mutants that lack these eleven putative hybrid HKs using targeted gene replacement. Upon constructing these mutants I will perform cellular and developmental phenotypic analysis of the mutants to determine the functions of these genes. Additionally, multiple-gene knockout strains will be produced of phylogenetically-related genes in cases where no phenotype is observed. I have produced four homokaryotic knockouts (without a robust phenotype), and am in the process of generating double mutants of the two HKs which have the highest relatedness in their sensor domains.

The phenotypes of HK mutants can be compared to the HPT, and the RR mutants. Analysis of one of the RR, *rrg-1*, has begun. One or more of the HKs are likely to regulate RRG-1. Analysis of the HKs and RRs will help provide insight into two-component signal transduction pathways and may contribute to the development of new antifungal agents for mammalian and plant pathogens.

409. One of the two Dicer-like proteins in the filamentous fungi *Magnaporthe oryzae* genome is responsible for hairpin RNA-triggered RNA silencing and related siRNA accumulation. Naoki Kadotani, Hitoshi Nakayashiki, Yukio Tosa, Shigeyuki Mayama. Laboratory of Plant Pathology, Kobe University, Kobe, Japan.

Dicer is an RNase III-like enzyme playing a key role in the RNA silencing pathway. Genome sequencing projects have demonstrated that eukaryotic genomes vary in the numbers of Dicer-like (DCL) proteins from one (human) to four (Arabidopsis). Two DCL genes, Magnaporthe Dicer-like (MDL)-1 and -2, have been identified in the genome of the filamentous fungus *Magnaporthe oryzae* . Here we show that the knockout of MDL-2 drastically impaired gene silencing of enhanced green fluorescence protein (eGFP), by hairpin RNA, and reduced related siRNA accumulation to non-detectable levels. In contrast, mutating the other DCL, MDL-1 exhibited a gene silencing frequency similar to wild-type and accumulated siRNA normally. The silencing-deficient phenotype and loss of siRNA accumulation in the mdl-2 mutant was restored by genetic complementation with the wild-type MDL-2 allele. These results indicate that only MDL-2 is responsible for siRNA production and no functional redundancy exists between MDL-1 and MDL-2 in the RNA silencing pathway in *M. oryzae*. Our findings contrast a recent report in the filamentous fungus *Neurospora crassa* where two DCL proteins are redundantly involved in the RNA silencing pathway but are similar to the results obtained in a more distantly related organism *Drosophila melanogaster* where an individual DCL protein has a distinct role in the siRNA/miRNA pathways.

410. In vitro analysis on the assembly mechanisms of the *Aspergillus* CCAAT-box binding factor. Masashi Kato and Tetsuo Kobayashi. Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.

The CCAAT-box is one of the most common cis-elements present in the promoter regions in eukaryotes. It has been shown to be important in the high-level expression of many genes in *Aspergillus* species. The factor which binds to the CCAAT-box, so-called the Hap complex, belongs to the NF-Y family and consists of three subunits, HapB, HapC and HapE. Previously we have shown that the number of HapE is strictly dependent on the number of HapE, suggesting the number of HapC could adjust that of HapE by forming stable heterodimers prior to assembly of the Hap complex[1)]. In this study, we performed a reconstitution study with the recombinant subunits and ^{35}S-labeled in vitro translated subunits. Significant amounts of the translated HapE were recovered in the insoluble fraction while the other two subunits were in the soluble fraction. However, HapE was recovered in the soluble fraction when the recombinant HapC was added in the translation reaction. These results suggest that the HapC subunit plays a chaperon-like role specific to the HapE subunit. Furthermore, pull-down assays of the labeled subunits with recombinant GST or MBP fusion subunits were also carried out to investigate the mode of subunit assembly of the *Aspergillus* Hap complex.
1) M. Kato et al. FEBS Lett. 512, 227-229 (2002)

411. Specific distribution in basidiomycete *Lentinula edodes* hymenophore of the transcripts of various *L. edodes* genes. S. Katsukawa[1], S. Kaneko[2], Y. Tanaka[1], Y. Sakuragi[1], T. Yamazaki[1], Y. Miyazaki[3] and K. Shishido[1]. [1)]Department of Life Science, Tokyo Institute of Technology, Yokohama, Japan; [2)]Mitsubishi-Kagaku Institute of Life Sciences, Tokyo, Japan; [3)]Department of Applied Microbiology, Forestry and Forest Product Research Institute, Tsukuba, Japan.

By using in situ RNA-RNA hybridization, we analyzed the distribution in *Lentinula edodes* hymenophore of the transcripts of several *L. edodes* genes, obtaining the following results. (1) Large amounts of the transcripts of ribonucleotide reductase small subunit gene (*rnr2*) and UMP-CMP kinase gene (*uck1*) are present in both hymenium and outer region of trama.The hymenium is the part for production of basidiospores and the outer region of trama is the region branching out into subhymenium (on the top of which hymenium is formed). (2) The ras transcript is present mostly in outer region of trama, while the transcript of trimeric G-protein alpha-subunit gene (*ga1*) is mostly in hymenium. The results suggest that *rnr2* and *uck1* genes play a role mainly in the nucleotide biosynthesis essential for production of basidiospores and for divergence of trama cells into subhymenium cells. The ras and *ga1* play a role in divergence of mycelial cells and in spore-production respectively. Analysis of the transcripts of RecQ-type DNA helicase gene (*recQ*) and other developmental regulated genes is in progress.

412. Loss-of-function mutations in the *Aspergillus nidulans xprG/phoG* gene are associated with the loss of extracellular protease activity and a repressible acid phosphatase. Margaret E. Katz, Karen-Ann Gray, Stella M.H. Bernardo and Brian F. Cheetham. Molecular and Cellular Biology, University of New England, Armidale, NSW AUSTRALIA,

Two types of mutations in the *A. nidulans xprG* gene have been identified. The *xprG1* mutation is semi-dominant and associated with increased extracellular protease activity, decreased ability to utilise some nitrogen sources and dark conidia. Strains carrying the recessive *xprG2* mutation are protease-deficient and have pale conidia. The *xprG* gene is tightly linked to *sarB*, a gene involved in nitrogen metabolism. A clone containing the *phoG* gene was isolated by complementation of the *xprG2* mutation. To confirm that *phoG* and *xprG* are allelic, a *phoG-* knockout was generated and proved to have a phenotype indistinguishable from the *xprG2* mutant. Sequence analysis showed that *xprG1* is a missense mutation while *xprG2* is a frameshift mutation in the 9th codon of the gene. The *xprG/phoG* gene did not complement the *sarB7* mutant and no mutations were found in the *xprG* coding region of a *sarB7* mutant. It has been reported that the *phoG* gene encodes or regulates nonrepressible acid phosphatase activity in *A. nidulans* and mutations in a *N. crassa* homologue, *vib-1*, show reduced nonrepressible acid phosphatase activity. In contrast, we have shown that loss-of-function mutations in the *xprG/phoG* gene are accompanied by the loss of a phosphate-repressible acid phosphatase.
The *xprF* and *hxkC* genes encode atypical hexokinases involved in the regulation of extracellular proteases. The *xprG2* mutation suppresses loss-of-function mutations in the *xprF* and *hxkC* genes. XprF, HxkC and XprG contain putative nuclear localisation sequences and gfp-tagged XprF has been shown to be located predominantly in the nucleus. The yeast two-hybrid system is being used to test the hypothesis that the *xprF* and *xprG* genes encode interacting proteins.

413. Functional Analysis of Aspergillus nidulans alpha-COP. Eun-Joung Song, Ki-Hyun Kim and Hee-Moon Park. Chungnam National University, Korea

Our previous results showed that a mutation in alpha-COP, component of COPI vesicle, is responsible for the temperature dependent osmo-sensitive phenotype of Aspergillus nidulans and Saccharomyces serevisiae, which is mainly due to the defect in cell wall biogenesis. In S. cerevisiae the interaction between alpha-COP and alpha-COP is required for the functional COPI vesicle and thus for normal cell wall biogenesis. We cloned a gene for a alpha-COP/SodVICp interactor, alpha-COP, in A. nidulans using Yeast Two-Hybrid Screening. Interacting domain analyses between two proteins revealed that each C-terminal domain of alpha-COP and alpha-COP plays a crucial role in their interactions. Especially, 283-288 region of alpha-COP was essential. It was also confirmed that the N-terminal WD40 domain of alpha-COP plays as cis-acting element in interaction with alpha-COP mediated by the C-terminal domain of alpha-COP as the previous results of S. cerevisiae. We also showed that temperature dependant osmo-sensitive phenotype of alpha-COP mutant can be rescued by the alpha-COP overexpression which stabilizes alpha-COP mutant.

414. Inducer-dependent nuclear localization of a $Zn(II)_2Cys_6$ transcriptional activator, AmyR. Tetsuo Kobayashi and Masashi Kato. Department of Biological Mechanisms and Functions, Nagoya University, Nagoya, Japan

AmyR is a $Zn(II)_2Cys_6$ transcriptional activator that regulates expression of the amylolytic genes in *Aspergillus* species. Subcellular localization studies of GFP fused AmyR revealed that the fusion protein preferentially localized to the nucleus in response to isomaltose, the physiological inducer of the amylolytic genes. The C-terminal domains of AmyR, designated as MH3 (residues 419-496) and MH4 (residues 516-542), were essential for sensing the inducing stimulus and regulating the subcellular localization, while the nuclear localization signals were identified within the N-terminal $Zn(II)_2Cys_6$ DNA binding motif. The MH2 domain (residues 234-375) located in the middle of AmyR was required for transcriptional activation of the target genes. Cytoplasmic localization of AmyR under non-inducing conditions does not seem to be caused by masking of the AmyR nuclear localization signal (NLS), since addition of an unrelated NLS to the NLS-mutated AmyR derivative retained isomaltose-dependent nuclear localization.

415. Post-translational regulation of AreA, the global transcriptional activator of nitrogen metabolism in *Aspergillus nidulans*. Koon Ho Wong, Richard B. Todd, Meryl A. Davis and Michael J. Hynes. Department of Genetics, University of Melbourne, Australia.

In *Aspergillus nidulans*, the global nitrogen regulatory gene *areA*, encoding a positively acting GATA transcription factor AreA, is required for activating genes involved in nitrogen metabolism. When a good nitrogen source like ammonium or glutamine is available, *areA*-dependent genes are expressed at a low level, while the expression of these genes is up regulated when only a poor nitrogen source, like alanine, is present (nitrogen limiting condition). This differential expression is controlled by *areA* transcript stability and the interaction of AreA with a negative regulator NmrA. A further increase in gene expression, which is not mediated by these two regulatory mechanisms, is observed under nitrogen starvation and correlates with AreA accumulation in the nucleus. Nuclear accumulated AreA is rapidly exported upon addition of a nitrogen source via the CrmA exportin. We therefore sought to investigate the importance of post-translational modifications on nuclear localization and function of AreA.
We have shown that AreA is multiply phosphorylated and its phosphorylation status differs under nitrogen sufficient, limiting or starvation conditions. A number of conserved potential phosphorylation sites on AreA have been mutated and assessed for their roles in AreA function. In addition, AreA contains a highly conserved *s*mall *u*biquitin-like *mo*difier (SUMO) modification site adjacent to a putative CrmA exportin binding motif. We have deleted the gene encoding the SUMO peptide and mutated the putative sumoylation site on AreA to address the involvement of sumoylation in the regulation of AreA.

416. Contribution of ammonia to the expression of *pel*B and PL secretion during pathogenicity of *C. gloeosporioides*. H.Kramer-Haimovich[1], E.Servi[1], Y.Okon[2], T. Katan[1], J. Rollins[3] and D. Prusky[1]. [1]Department of Postharvest Science of Fresh Produce, ARO, The Volcani Center, Bet Dagan 50250, [2]Department of Plant Pathology and Microbiology, Hebrew University, Faculty of Agriculture, Rehovot 76100, Israel, [3] Department of Plant Pathology, University of Florida, Gainesville, Florida 32611, USA.

Tissue alkalinization resulting from the accumulation of ammonia predisposes avocado fruit to attack by *C. gloeosporioides* by enhancing transcript expression of *pel*B and the secretion of pectate lyase a virulence factor expressed during fungal colonization. However recently obtained data suggests that nitrogen also affects *pelB* expression. Natural pH environment occurring in the fruit triggers the initiation of ammonia secretion, its accumulation at the infection court and the increase of the host pH during the necrotrophic attack. Ammonia secreted induced PL secretion by the pathogen. *C. gloeosporioides* nit- mutants lacking the capability to secret ammonia, could not secret PL at inducing pH conditions and showed reduced pathogenicity. However, infiltration of avocado fruits with ammonia enhanced *C. gloeosporioides* attack. Present results suggest that the natural pH changes occurring in the host tissue during ripening, may induce the secretion of ammonia by the pathogen, and the ammonification and alkalinization of tissue both together contributes to the expression of *pel*B, secretion of PL and decay development.

417. Analysis of differential gene expression across regions of the *Aspergillus oryzae* mycelium. Kumiko Masai[1], Jun-ichi Maruyama[1], Harushi Nakajima[2], Kazutoshi Sakamoto[3], Osamu Akita[3], Katsuhiko Kitamoto[1]. [1]Department of Biotechnology, University of Tokyo, Tokyo, Japan. [2]Department of Agricultural Chemistry, Meiji University, Kawasaki, Japan. [3]National Research Institute of Brewing, Higashi-Hiroshima, Japan.

Regions of differentiation in filamentous fungi mycelia can be observed during growth on solid substrates. This suggests that each of these regions may have different functions during the life cycle of the fungus and accordingly, their characteristics being controlled by gene(s) specifically expressed in each region. Here, we propose a mycelial-scale gene expression analysis approach to determine the gene(s) responsible for this phenomenon in the industrially important filamentous fungus *Aspergillus oryzae*. Upon observation of a mycelium of *A. oryzae*, three distinct regions were detected. They were designated as tip, white and basal, based on their visual characteristics. Tip cells growing at the edge of the colony comprised the tip region, whereas aerial hyphae and developed conidiophores were observed in the white and basal regions, respectively. Differential expression of genes in the three regions was detected by RT-PCR in the preliminary analysis. Microarray analysis using the *A. oryzae* cDNA chip was performed to identify the genes that are specifically expressed in each region. Genes involved in the translation process were abundant in the tip region, whereas, genes encoding transporters were expressed at high levels in the basal region. This result supports the assumption that the genes expressed in each region reflect the characteristics observed in each region. Thus, this study has laid the basic foundation in the investigation of what makes each region unique. Further characterization of each region and functional analysis of selected genes will be presented.

418. The MpkB MAP kinase plays a central role in signaling for the sexual development of *Aspergillus nidulans*. Sei-Jin Lee[§1], Ji-Young Kang, Mira Jin, Dong-Min Han[2], Keon-Sang Chae and Kwang–Yeop Jahng[*1]. Division of Biological Sciences, Institute for Basic Sciences, Institute for Molecular Biology and Genetic Engineering, [1]Korea Basic Science Institute Jeonju Center, Chonbuk National University, Chonju 561-756, Republic of Korea, [2]Division of Life Sciences, Wonkwang University, Iksan 570-749, Republic of Korea

Two genes encoding a MAP kinase homologue, designated as *mpkB* and *mpkC*, were isolated from *Aspergillus nidulans* by PCR with degenerated primers. The amino acid sequence of MpkB showed 92% - 93% identity to the MAP kinases of diverse phytopathogenic fungi that are involved in differentiation and pathogenicity. The amino acid sequence of MpkC showed 77% - 85% identity to the stress activated protein kinases (SAPKs) of filamentous fungi that play a role in responding to environmental stresses. Deletion of the *mpkB* gene caused failure in forming the sexual organ cleistothecium in any condition that could induce sexual development, suggesting that MpkB may be essential for transmitting the signals for sexual development. In addition, deletion mutant of *mpkB* showed a slower hyphal growth and an aberrant conidiophore morphology. Deletion and over-expression mutants of *mpkC* showed no detectable phenotypes under any conditions provided by various external stresses. Although the *mpkB* and *mpkC* genes were constitutively transcribed through entire life span, transcripts of both genes showed varieties in size and abundance according to the developmental stages. The *mpkB* transcript was hardly detected in the deletion mutant of *veA* in contrast to highly accumulated in the over-expression mutant of *veA*. Deletion of *mpkB* caused a drastic reduction of *medA* and *steA* transcript accumulation during sexual reproduction as well as the *stuA* transcript during both asexual and sexual developments. Taken together these results, we propose that the MpkB MAP kinase has a central role in diverse signaling pathways that mediate sexual development in *A. nidulans*.

419. A MAP Kinase Pathway Essential for Mating and Contributing to Vegetative Growth in *Neurospora crassa*. Dan Li, Piotr Bobrowicz, Heather H. Wilkinson and Daniel J. Ebbole. Department of Plant Pathology and Microbiology, Texas A & M University, College Station, Texas 77843, United States

MAP kinases homologous to *Saccharomyces cerevisiae* Fus3p/Kss1p have been identified in several plant pathogenic fungi and found to be required for pathogenicity and sexual reproduction. To better understand the role of MAP kinase signaling in development in *Neurospora crassa*, and to identify downstream target genes of the pathway, we isolated, cloned, and disrupted the FUS3 homolog *mak-2* and the STE12 homolog, *pp-1*. The *mak-2* and *pp-1* deletion mutants have reduced growth rate, produce short aerial hyphae, and fail to develop protoperithecia. In addition, ascospores carrying null mutations of either gene are inviable. Subtractive cloning was used to isolate several genes that display reduced expression in the *mak-2* mutant. Expression of some of these genes is protoperithecia-specific and three of them are part of a gene cluster potentially involved in the production of a polyketide secondary metabolite. The ORF encoding a putative polyketide synthase in this gene cluster was partially deleted and phenotype of the mutant was carefully studied. Microarray analysis was used to extend the analysis of gene expression in *mak-2* and *pp-1* mutants. The role of the MAP kinase pathway in both sexual and asexual development as well as secondary metabolism is consistent with the dual regulation of the mating process and pathogencity/invasive growth observed in many fungal pathogens.

420. Cold induced gene expression in *Botrytis cinerea*. Amnon Lichter, Ayelet Ezra, H-W. Zhou, Orit Dvir and Anna Danshin. ARO, The Volcani Center, Israel

Cold storage is the major postharvest tool to prevent fungal decay. As evident from its potential to develop on grapes during extreme cold storage and cause gray mold, *Botrytis cinerea* is well adjusted to low temperature. This condition may require optimization of cellular processes as well specialized functions which are redundant at high temperature. The growth kinetics of *Botrytis cinerea* were determined at a temperature of zero compared to 20C, in different media and with and without conidial germination at zero. We have approached the mechanism of cold fitness by molecular cDNA subtraction following exposure of germinating spores to low temperature. A subset of clones obtained by this procedure were sequenced and screened for differential expression at low or high temperature using cDNA probes. A subset of those clones were verified to be differentially expressed by northern analysis. The limited number of clones with known sequence homology permit a first glance into molecular mechanisms that makes this fungus a successful postharvest pathogen.

421. Identification of genes differentially expressed in response to acidic induction in *Trichophyton rubrum*. Henrique C.S. Silveira, Mônica S. Ferreira-Nozawa, Antonio Rossi and Nilce M. Martinez-Rossi. Universidade de São Paulo, FMRP, Ribeirão Preto, Brazil.

Trichophyton rubrum, a filamentous fungus that causes infections in human skin and nails, is recognized as cosmopolitan and is one of the most frequently encountered dermatophytes. Although several factors contribute to the pathogenicity of dermatophytes, the successful initiation of infection depends on the capacity of the infecting fungus to sense and overcome the acidic pH of the skin. Thus, it is important to understand the metabolic responses that govern homeostatic and extracellular pH sensing in dermatophytes. Here, we describe genes differentially expressed in response to acidic pH by employing the suppression subtractive hybridization (SSH) approach. The tester and driver cDNAs were obtained from mycelium respectively derepressed at pH 5.0 and at pH 8.0. Out of a total of 300 cDNA clones analyzed by dot-blot macro-arrays, we confirmed 96 clones differentially expressed at pH 5.0, which represented 21 different genes. Based on BLAST homology, the clones selected from these libraries are largely of fungal origin. The hypothetical proteins represent 57% of the total, and the putative proteins identified are involved in metabolism, cell differentiation, cell cycle, and resistance mechanisms. These studies should provide data that will be useful for identifying the metabolic machinery essential for initiating infection by *T. rubrum*.
Financial support: FAPESP, CNPq, FAEPA and CAPES.

422. Identification of potential regulatory RNAs. Tami McDonald, Zhihong Zhang, and Fred Dietrich. University Program in Genetics, Duke University, Durham, North Carolina USA.

Gene regulation is accomplished in fungi by several mechanisms. One intriguing class of gene regulators is comprised of regulatory RNAs. There are three main mechanisms of regulation by regulatory RNAs: Promoter competition, in which two nearby or overlapping promoters compete for the polymerase or transcription factors; Transcription interference, in which transcription of an upstream RNA facilitates read-through across the promoter of the downstream gene; and RNA-mediated control in which RNA transcripts bind to regions to prevent transcription. We are investigating the use of 5' SAGE to identify additional small RNA species in fungi. This method allows high throughput identification of transcription start sites. While this method is used primarily for identification of the 5' transcription start sites of protein coding genes, we have also observed start sites corresponding to transcription of what are possibly small, previously un-identified RNA molecules. We are investigating several of these potential regulatory molecules, including one in the upstream region of the mitochondrial transporter ODC2, suggesting an upstream microORF, and several tags clustering on the strand of DNA complementary to ASE1, a cell-cycle regulated microtubule-associated protein (MAP) found at spindle fibers, suggesting a reverse microORF. Preliminary northern data suggests that a RNA product is formed by microORF upstream of ODC2.

423. Expression and characterization of *Trichoderma reesei* beta-xylosidase in *Aspergillus oryzae*. Sandra T. Merino, Hanshu Ding, and Joel R. Cherry, Novozymes Biotech, Davis, CA.

L-Arabinose residues, in furanose form, are widely distributed in plant tissue heteropolysaccharides, such as arabinans, arabinogalactans and arabinoxylans. The presence of arabinose as side groups can restrict enzymatic hydrolysis of hemicellulose. The *T. reesei* beta-xylosidase has been shown to have alpha-L-arabinosidase activities against small synthetic model substrates (Margolles-Clark et al., 1996, Applied and Environmental Microbiology, Vol. 62, No. 10, p. 3840-3846). We describe the cloning and expression of the *T. reesei* beta-xylosidase gene in the host, *Aspergillus oryzae*. Broth samples were analyzed for activity and the results show activity on the substrate xylan and even higher activity on p-nitrophenyl-xylose when compared with a positive control.

424. The *Aspergillus niger* Unfolded Protein Response Element. Harm Mulder, Igor Nikolaev and Susan Madrid. Danisco Innovation, Copenhagen Denmark.

Eukaryotic cells respond to the accumulation of unfolded proteins in the ER by activating a pathway known as the unfolded protein response (UPR), that culminates in the induction of a set of genes with functions affecting nearly every stage of the secretory pathway. The promoters of UPR target genes contain an unfolded protein response element (UPRE), which confers the stress inducibility to the gene, via an interaction with the transcription activator HACA. In the promoters of the ER-stress responsive genes *bipA, cypB, pdiA, prpA, tigA* and *hacA* a consensus sequence was identified, which was located close to the transcription start site of the gene (in vitro selection procedure, an optimal binding site for HACA was isolated. This sequence, ACACGTGTCCT, resembles the UPRE but lacks the spacer nucleotide, and *in vivo* it behaves as a more potent *cis*-acting element. Two UPREs were identified in the upstream region of the *hacA* gene itself, an interesting finding given the fact that ER stress is associated with the appearance of shorter *hacA* transcript. The mechanism behind this truncation of the *hacA* mRNA upon ER stress is still unknown. However, the presence of those two UPREs could indicate a possible role for HACA itself in its transcriptional regulation under ER stress, a hypothesis we are currently investigating.

425. RNA silencing as a tool for exploring gene function in ascomycete fungi. Hitoshi Nakayashiki, Shugo Hanada, Nguyen Bao Quoc, Naoki Kadotani, Yukio Tosa, Shigeyuki Mayama. Laboratory of Plant Pathology, Kobe University, Kobe, 657-8501 Japan.

RNA silencing provides potentially versatile reverse genetic tools for exploring gene function in the post-genomics era. We have developed a pHANNIBAL-like silencing vector, pSilent-1, for ascomycete fungi, which carries a hygromycin resistance cassette and a transcriptional unit for hairpin RNA expression with a spacer of a cutinase gene intron from the rice blast fungus *Magnaporthe oryzae*. Application of pSilent-1 to two *M. oryzae* endogenous genes, MPG1 and polyketide synthase-like gene resulted in efficient silencing of the genes. RNA silencing was also induced by a pSilent-1-based vector in *Colletotrichum lagenarium* at a slightly lower efficiency than in *M. oryzae*, indicating that this silencing vector should provide a useful reverse genetic tool in ascomycete fungi.

426. Redundant and distinct roles of two PKA catalytic subunits in *Aspergillus nidulans*. Min Ni, Sara Rierson, Jeong-Ah Seo and Jae-Hyuk Yu. University of Wisconsin, Madison WI 53706 USA

Previously, PkaA, a cAMP-dependent protein kinase (PKA) catalytic subunit, was shown to play an important role in transducing a heterotrimeric G protein mediated vegetative growth signaling in *Aspergillus nidulans*. We have identified and characterized the *pkaB* gene encoding the second PKA catalytic subunit. Although the absence of *pkaB* alone did not cause clear phenotypic alterations, deletion of both *pkaA* and *pkaB* is found to be lethal, indicating that PkaB and PkaA constitute the sole PKA catalytic subunits. Overexpression of *pkaB* increased hyphal proliferation in wild type, and partially restored colony growth in *pkaA* deletion mutant, suggesting that PkaB also functions in vegetative growth signaling as a secondary PKA subunit. While an additional copy of *pkaB* rescued spore germination defects resulted from deletion of *pkaA* on glucose medium, it blocked spore germination in the medium without carbon source, indicating that PkaB may negatively regulate spore germination via sensing carbon sources. Furthermore, deletion of *pkaA* or up-regulation of *pkaB* resulted in the reduced tolerance of vegetative hyphae against oxidative stress, suggesting that PkaB and PkaA play opposite roles in responding to certain stresses. Taken together, we propose that PkaB is the secondary PKA catalytic subunit that plays a redundant role in vegetative growth signaling, but a distinct role in regulating germination, development, and stress response in *A. nidulans*.

427. Gene expression upregulated by a conidiogenesis patterning regulator Acr1 in *Magnaporthe grisea*. Marie Nishimura. National Institute of Agrobiological Sciences, Tsukuba, 305-8602, JAPAN.

Rice blast disease, caused by the filamentous fungus *Magnaporthe grisea*, is one of the most devastating diseases to global rice production. To understand the regulation mechanism of conidiation in *M. grisea*, a morphological mutant, *acr1*, has been studied. Acr1 is a functional homolog of MedA, a conidiophore-developmental regulator in *Aspergillus nidulans*. The *acr1* mutants produce chains of elongated conidia in a head-to-tail array, while the wild type conidia are produced in a sympodial array. In *acr1*, appressoria production is greatly reduced and the appressoria are defective in plant penetration. The reductions in the appressorium formation and penetration rates in the mutant are not complemented by the addition of exogenous cAMP. To investigate the genes upregulated by Acr1, an oligo DNA microarray, and nylon membranes arrayed with subtraction libraries constructed from conidial mRNA of *acr1* and its isogenic wild-type strain Guy11 were used. Results from these analyses were further confirmed by RT-PCR. The expression level of *Saccharomyces cerevisiae PCL1* - like G1 cyclin was reduced in *acr1*. Expression of several genes related to secondary metabolism were also controlled by Acr1. Recent progress on analysis of these genes will be presented.

428. Rho1 in *Ustilago maydis* affects mating, cell morphology, and perhaps viability. Zhanyang Yu and Michael H. Perlin*. University of Louisville, Louisville, KY 40292, USA

The highly conserved Rho/Rac small GTPase family regulates multiple signal transduction pathways involved in cell morphogenesis, cell cycle, cell-cell communication, and mating response. In *U. maydis*, the pathogen of maize, at least 25 putative members of this family have been identified. Several of these have been well characterized for their roles in cell separation or cell polarity (e.g., Cdc42 and Rac1). Others have been shown to be significant participants in regulating filamentous growth, pheromone response, and pathogenicity (i.e., Ras1 and Ras 2). Less is known about the function of the Rho homologues in this organism. In the fission yeast, *S. pombe*, Rho1 is essential for cell viability and cell polarity. From *U. maydis* we cloned *uro1*, encoding a Rho1 homologue. The genomic version contained a 400-bp intron relative to the cDNA coding region of 950 bp. The predicted amino acid sequence had 80% identity with Rho1 of *S. pombe*, as well as 47.9% and 49% identity with the Cdc42 homologues from *S. cerevisiae* and *U. maydis*, respectively. This identity was particularly strong in the two highly conserved GTP binding/hydrolysis motifs. The cDNA fragment was expressed in wild-type *U. maydis* strains and a variety of mutant strains. Over-expression of *uro1* reduced the mating efficiency on YPD-charcoal plates; the pheromone response was also reduced, especially for a2 cells, where it is normally the greatest. Attempts to disrupt the *uro1* gene were unsuccessful for either a1 or a2 haploid strains. On the other hand, successful disruption of one copy of the gene was achieved in two different diploid strains. This suggests that the *uro1* gene, in addition to its role in regulating mating, may also be required for viability. Moreover, disruption of a single copy of *uro1* in a diploid strain produced a dominant negative phenotype, eliminating the fuz reaction on charcoal media.

429. Slave Oscillators in Neurospora's Circadian Clock: a Role for RNA-binding Proteins? Nathan Price-Lloyd & Christian Heintzen. University of Manchester, Lancashire, UK

Metronomic changes in the physical environment have led to the evolution of correspondent molecular clocks. Oscillating with a period of approximately 24 hours, circadian clocks allow pre-adaptation to daily rhythmical changes concurrent with the Earth's rotation. In 1960, a hierarchical system of ‘master' and ‘slave' oscillators was proposed by Pittendrigh, whereby a central clock can relay information via a number of subordinate cogs to a variety of outputs. This model allowed for increased plasticity in clock-generated signal pathways, and the option to evolutionarily tinker with downstream components, bypassing the central clock.
Increasing evidence suggest that *Neurospora's* clock functions via such a model. Importantly, some plant RNA-binding proteins (RBPs) have been shown to act as subordinate oscillators, with evidence from fruit-flies and humans also showing circadian regulation of RBPs.
We BLASTed the *Neurospora* on-line database with the conserved regions of the plant, fruit-fly, and human RBPs, and recognised 43 *Neurospora* RBPs. One is of known biochemical function, one is putatively homologous to the plant RBPs, and several others have defined functional domains. We have begun characterising transcript levels in terms of temporal expression and reaction to *zeitgebers*. Prime candidate RBPs have been knocked out of the genome by homologous gene replacement. Rhythmic analysis of phenotype will be presented.

430. Regulation of cuticle and cell wall-degrading enzyme expression in *Fusarium oxysporum* . F.J. Calero-Nieto, A.L. Martínez-Rocha, A. Di Pietro, C. Hera & M.I.G.Roncero. Departamento de Genetica, Universidad de Cordoba, Campus Rabanales Ed. C5, 14071Cordoba, Spain

Degradation of different plant polymers by fungal pathogens requires the coordinated action of numerous hydrolytic enzyme activities. Vascular pathogens such as *Fusarium oxysporum*, have to overcome primary physical barriers of the plant cell wall in order to reach the vascular elements and to accomplish their rapid colonization. On the other hand, leaf pathogens have to degrade cutin, an insoluble lipid polyester matrix covering the surface of all aerial parts of plants. The implication of cell wall-degrading enzymes in vascular wilt disease caused by *F. oxysporum* has been suggested during penetration of the different layers of the root cortex to reach the vascular system and during colonization of the host by spreading upward through the xylem vessels. Whereas the role of cutin degrading enzymes in vascular wilt disease is currently unknown. Previous studies showed that *F. oxysporum* secretes an array of extracellular degradative enzymes capable of hydrolyzing different components of the plant cell wall and cuticle, including polygalacturonases, pectate lyases, xylanases and cutinases. Targeted disruption of individual corresponding structural genes showed no significant reduction in virulence of *F. oxysporum* on tomato plants, possibly due to functional redundancy. In order to elucidate the role of xylanases and cutinases during the pathogenesis of *F. oxysporum*, and to establish their common regulatory mechanisms, we have identified and characterised two transcription factors, XlnR and Ctfa, known to regulate the expression of fungal xylanase and cutinase genes, respectively. Two types of mutants have been created by genetic transformation of *F. oxysporum* f.sp. *lycopersici* wild type strain 4287: loss-of-function mutants (DxlnR and Dctfa) were created by targeted gene replacement, and mutants harbouring a constitutively expressed allele of *ctfa* and *xlnR* were obtained by fusing the coding regions to the *gpdA* promoter. Conidiation, growth rates and virulence behaviour of these mutants on tomato plants were similar to those of the wild type strain. The expression level of xylanase and cutinase structural genes have been determined by real time quantitative PCR. Total extracellular xylanase and esterase activities were also determined for each pair of mutants.

431. The calpain-like protease PalB from *A. nidulans* is required for retarding the proteolytic processing of transcription factor PacC. Carlos J. Ono, Mônica S. Ferreira-Nozawa, Nilce M. Martinez-Rossi and Antonio Rossi. Universidade de São Paulo, FMRP, Ribeirão Preto, Brazil.

The conserved PacC signal transduction pathway mediates many metabolic events involved in ambient pH sensing in *A. nidulans*, and it is widely accepted that it governs only the response to neutral-to-alkaline pHs. The *pac*C gene codes for a Zn-finger transcription factor whose transcription is itself induced under alkaline growth conditions. The *pal* genes (*pal*A, B, C, F, H, and I) are putative members of a signaling cascade, whose function is presumed to promote the proteolytic activation of PacC. Thus, the current model states that the full-length version of PacC ($PacC^{72}$) is activated at alkaline pH by two sequential proteolytic steps that remove the C-terminal negatively acting domain. The conversion of $PacC^{72}$ to $PacC^{53}$ is PalB- and pH-dependent, whereas the conversion of $PacC^{53}$ to $PacC^{27}$ is pH-independent. It is assumed that the $PacC^{27}$ processed form is perhaps the sole functional form of PacC at alkaline pH. This model implies that inactivation of any of the *pal* genes should lead to an abundant expression of $PacC^{72}$ irrespective of the extracellular pH. To test this hypothesis we purified PacC as briefly described here: the truncated PacC protein, which contains the three zinc-fingers of gene *pac*C, was expressed in *E. coli*, purified by affinity chromatography, and polyclonal antibodies were raised in albino male rabbits. The anti-PacC antibodies was purified, chemically cross-linked to hydrazide-Sepharose resin, and used for purification of PacC by immunoaffinity chromatography. We present evidence that proteolytic processing of $PacC^{72}$ occurs earlier in the *pal*B7 mutant grown under acidic conditions. Furthermore, proteolysis of PacC is extensively detected in the *pal*B7 mutant grown under alkaline conditions, where $PacC^{72}$ should be abundant. Thus, the full-length version of PacC was abundantly detectable only in strains in which *pal*B is functional.
Financial support: FAPESP, CNPq, FAEPA and CAPES.

432. *Aspergillus oryzae atfB* encodes a transcription factor, which is required for stress tolerance of conidia. Kazutoshi Sakamoto. NRIB, Higashi-Hiroshi, Japan

In the solid-state culture, *Aspergillus oryzae* exhibits phenotypes such as the high production of enzymes, conidiophore development, and the production of various kinds of secondary metabolites. Though these characteristics should involve various gene expressions, the only a few regulatory systems have been understood. From the EST database of *A. oryzae*, we found two genes encoding transcription factors that show high homology to *atf1* of *Shizosaccharomyces pombe* and named *atfA* and *atfB* respectively. The *atfB* gene was upregulated at the late phase of solid-state culture, while *atfA* gene was constitutively expressed. We tried to identify *atfB* target genes by microarray analyses. In the *atfB*-overexpression strain, about 30 genes including catalase gene (*catA*) were upregulated. Almost all of these genes were upregulated at the late phase of solid-state culture in wild type strain. Furthermore the expression of these genes were downregulated in the *atfB*-disrupted strain. The disruptant strain grew normaly but its conidia lost several stress tolerances.

433. Characterization of lentinan degrading exo-glucanase-encoding gene *exg2* from *Lentinula edodes*. Y. Sakamoto[1], K. Minato[2], S. Kawakami[2], M. Mizuno[2], T. Irie[1] and T. Sato[1] [1]Iwate Biotechnology Research Center, Iwate, Japan. [2]Department of Agriculture, Kobe University, Hyogo, Japan

Lentinan, which is a beta-1, 3-linked-D-glucan with beta-1, 6 branches isolated as anti-tumor active-substrate from *Lentinula edodes*, is purified from fresh fruit-bodies and marketed for clinical use. However, it is known that lentinan content decreases during post-harvest preservation. Lentinan contents were measured by ELISA using anti-lentinan anti-bodies, and it was revealed that lentinan degradation is mainly occurred in gill. We also revealed that beta-glucanase activity increased during post-harvest preservation. Then we cloned two exo-glucanase encoding genes, *exg1* and *exg2* from *L. edodes*. The *exg1* gene had 1.2 kbp cDNA length, and calculated molecular weight and pI value of the encoded protein was 44 kDa and 4.36, respectively. Putative amino acid sequence of the *exg1* displayed 67% identity to *AbEXG1* of *Agaricus bisporus*. Two conserved glutamic acids that are within the catalytic active site in yeast exo-beta-1, 3-glucanase-encoding genes were conserved in *exg1* of *L. edodes*. The *exg2* gene had 2.4 kbp cDNA length, and calculated molecular weight and pI value of the encoded protein was 79 kDa and 4.66, respectively. Putative amino acid sequence of the *exg2* displayed 40% identity to *exgS* of *Aspergillus saitoi*. The *exg2* gene had two imperfect copies of motif that are conserved in ascomycetous exo-glucanases. Transcription levels of the *exg1* and *exg2* genes were higher in stipe than that in pileus of young fruiting bodies. Transcription level of the *exg1* gene decreased, but the *exg2* gene increased after harvest. Western blot analysis showed that EXG2 protein expression increased after harvest. Purified EXG2 protein degraded lentinan, therefore, we concluded that the *exg2* gene is a lentinan degrading exo-glucanase encoding gene.

434. Protein Production and Unfolded Protein Response in Fermentations of *Trichoderma reesei* and its Transformant Expressing Endoglucanase I with a Hydrophobic Tag. Anna Collén[1], Michael Bailey, Markku Saloheimo, Merja Penttilä and Tiina Pakula. VTT Biotechnology, P.O. Box 1500, FIN-02044 VTT, Finland. [1]Department of Biochemistry, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, S-221 00 Lund, Sweden. Present address: AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden

The effect of induction of protein production was studied in bioreactor cultures of the *T. reesei* strain Rut-C30 and its transformant expressing endoglucanase I (EGI, Cel7B) fused with a hydrophobic tag. The peptide tag was previously designed for efficient purification of the fusion protein in aqueous two-phase separation. The first phase of the bioreactor cultivations was carried out on glucose containing minimal medium. At the stage when glucose was nearly depleted, the medium was supplemented with rich medium containing lactose as a carbon source to induce production of cellulases. The transformant produced somewhat less secreted protein and cellobiohydrolase I (CBHI, Cel7A) activity than the parental strain. Western analysis of intracellular proteins showed that the fusion protein $EGI_{CORE\text{-}P5}$(WP)4 accumulated inside the cell, indicating impaired secretion of the protein. Two-dimensional gel analysis suggested that the fusion protein was possibly trapped early in the secretory pathway. The mRNA levels of the UPR (unfolded protein response) target genes, bip1 and pdi1, and the level of the activated hac1 transcript encoding the UPR transcription factor, increased at the same time with an increase in the transcript levels of cellulase genes, suggesting UPR activation in response to cellulase induction. However, only a minor increase in pdi1 and bip1 transcript level was observed in the transformant expressing the fusion protein compared to its parental strain. In addition, slightly lower CBHI production and cbh1 mRNA levels were measured in the transformant as compared to the parental strain, indicating activation of the novel repression mechanism of genes encoding secreted proteins in response to secretion stress, RESS (repression under secretion stress).

435. ENVOY, a novel PAS/LOV domain protein, regulates cellulase gene transcription dependent on light and connects carbon source signaling to light response in *Hypocrea jecorina (anamorph Trichoderma reesei)*. Monika Schmoll[1], Lisa Franchi[2], and Christian P. Kubicek[1]. [1]Institute of Chemical Engineering, Vienna University of Technology, Getreidemarkt 9/1665, A-1060 Wien, Austria. [2]Universita' di Roma "La Sapienzia", Policlinico Umberto I, Viale Regina Elena 32, 00161 Roma, Italy

Envoy, a PAS/LOV domain protein with high similarity to the *Neurospora* light regulator VIVID and links cellulase induction by cellulose to light signaling. An $env1^{PAS-}$ mutant grows slower in the presence of light but remains unaffected in darkness as compared to the wild-type strain QM9414. *env1* transcription rapidly responds to a light pulse, this response being different upon growth on glucose or glycerol, and it encodes a regulator essential for *H. jecorina* light tolerance. Despite their similarity, *env1* could not compensate for the lack of *vvd* function. Induction of cellulase formation in *H. jecorina* by cellulose is enhanced by light in the wild-type strain as compared to constant darkness, whereas a delayed induction in light and an initially stronger induction of cellulases followed by a shut down of expression was observed in the $env1^{PAS-}$ mutant in constant darkness. Light does not lead to cellulase expression in the absence of an inducer.
Envoy thus connects light response to carbon source signaling and consequently light must be considered as an additional external factor influencing gene expression in this fungus.

436. New components of nitrogen regulation in *Fusarium fujikuroi*: the role of NPR1-Gf und CPC1-Gf. Birgit Schönig, Bettina Tudzynski. Westfälische Wilhelms-Universität Münster, Schlossgarten 3, 48149 Münster, Germany

The rice pathogen Fusarium fujikuroi produces gibberellins (GAs), a group of economically important phytohormones. The production of GAs is subject to nitrogen catabolite repression: the central regulator of nitrogen repression, AREA-Gf, had been shown to directly activate the transcription of 6 out of 7 biosynthetic genes under nitrogen limitation conditions. Besides homologues to other key elements of nitrogen regulation in Saccharomyces cerevisiae, we are interested in homologues of the NPR1 protein and the effector of cross pathway control in filamentous fungi, CPC1 and their influence on secondary metabolism.
The NPR1 protein kinase regulates sorting and stability of nitrogen permeases and, as shown recently, also the transport of the AREA homologue GLN3 into the nucleus. In order to show if the NPR1 homologue in Fusarium fujikuroi is involved in nitrogen regulation and GA production, we cloned and deleted the corresponding gene, npr1-Gf.
The central effector of cross pathway control in filamentous fungi, CPC1, has been shown not only to affect the availability of amino acids, but also secondary metabolism, stress response and pathogenicity. To examine if the orthologue in F. fujikuroi is involved in general secondary metabolism control under amino acid starvation, we cloned and deleted the genomic copy, cpc1-Gf. Macroarray experiments revealed several target genes of cpc1 not described in filamentous fungi before. The effect of gene replacement on GA production is under investigation.

437. Siderophore biosynthesis is negatively regulated by SreA in *Aspergillus fumigatus*. Markus Schrettl, Claudia Kragl, and Hubertus Haas. Department of Molecular Biology, Innsbruck Medical University, Austria.

Virtually all organisms require iron for their growth, because this metal is indispensable for various cofactors, e.g. heme moieties and iron-sulfur clusters. Induced by iron starvation, most fungi excrete siderophores - low molecular-mass ferric iron chelators - in order to mobilize extracellular iron. The filamentous ascomycete *A. fumigatus* produces two major siderophores: it excretes triacetylfusarinine C to capture iron and contains ferricrocin as an intracellular iron storage compound. Recently it was shown, that the siderophor system is absolutely required for virulence of this fungus and therefore regulation of this iron uptake system is of great interest. Inactivation of the GATA-type transcription factor SreA caused derepression of the reductive iron uptake system, of extracellular siderophore biosynthesis and accumulation of intracellular siderophores during iron-replete condition. Such mutants displayed increased sensitivity to the redox cycler menadione. Moreover, an increase of extracellular iron availability caused a decrease of radial growth in these mutants. These data suggest that deregulation of the iron uptake system leads to increased oxidative stress, presumably via Fenton/Haber-Weiss-chemistry. Taken together, the data show that SreA is a key regulator of iron uptake in *A. fumigatus* and gene profiling of the mutant will help to identify genes involved in iron metabolism.
This work was supported by Austrian Science Foundation.

438. Alternative initiation of translation and light-specific phosphorylation yield two forms of the essential light and clock protein White Collar-2. Carsten Schwerdtfeger, Jennifer J. Loros, and Jay C. Dunlap, Dartmouth Medical School, Genetics, Hanover, NH, USA

Blue light and temperature are important environmental cues and also play an important role in the *Neurospora crassa* circadian clock. To date, the core components of the circadian clock in *N. crassa* are WHITE COLLAR-1 (WC-1), WHITE COLLAR-2 (WC-2), and FREQUENCY (FRQ) proteins with several kinases, phosphatases, and a ubiquitin ligase also identified. WC-1 and WC-2 are both GATA-type zinc finger, DNA binding transcription factors with a nuclear localization signal and PAS (**P**er, **A**rnt, **S**im) dimerization domains (Ballario, et al. 1996, Taylor and Zhulin, 1999).
Here we demonstrate the detection of a short form of WC-2 (sWC-2), which emerges from the same transcript as long WC-2 (lWC-2), arising via alternative initiation. sWC-2 is missing ~15kDa from its N-terminus and migrates at around 45kDa. WC-2 antibodies show sWC-2 to be predominantly a nuclear protein that undergoes a hyperphosporylation in response to blue light. By generating a mutant that makes sWC-2 only we find the light-driven transcriptional responses of the *albino* genes and *frq* to be at normal levels. However, the circadian oscillation is abolished. Furthermore, the lack of lWC-2 leads to a change in light adaptation and the high light response similar to the *vvd (vivid)* mutant. Our results suggest different roles for lWC-2 and sWC-2 in clock and blue light signal transduction in *Neurospora crassa*.

439. The effect of viral infection on gene silencing in *Cryphonectria parasitica*. Gert C. Segers and Donald L. Nuss, UMBI-CBR, University of Maryland, College Park, MD 20742.

The C. parasitica / hypovirus system provides unique opportunities to study the effect of virus infection on gene silencing as well as antiviral responses in fungi. To induce gene silencing in *C. parasitica*, a vector (pGS) that allows expression of a hairpin construct was developed. pGS contains multiple cloning sites on either side of an intron from the *C. parasitica* GPD gene, the *A. nidulans* GPD promoter to drive expression and the *C. parasitica* GPD terminator to terminate transcription. Transformation of pGS containing EGFP (pGS-EGFP) into an EGFP expressing *C. parasitica* strain resulted in transformants of which app. 50% were silenced, as judged by monitoring EFGP fluorescence. EGFP-silenced strains showed severe reduction in EGFP transcript and protein accumulation. Subsequent infection of EGFP silenced strains with a hypovirus resulted in a moderate increase in EGFP transcript levels, suggesting a negative effect of virus infection on gene silencing.
Hypoviral protein P29 shares similarity with HC-Pro of potyviruses, a known suppressor of silencing in plants. Transformation of a *C. parasitica* strain expressing both EGFP and a p29 transgene with pGS-EGFP failed to silence EGFP. This suggests that P29 can preventively suppress gene silencing. Experiments are underway to determine the mechanism of P29 suppression of silencing.
Genes encoding two Dicer homologues (DCL1 and DCL2) and one Argonaute homologue (AGL1) have been isolated, and generation of corresponding null mutants is in progress. The effect of AGL1 gene deletion on viral accumulation will be determined.

440. Structural and functional analysis of *Cryptococcus neoformans* hybrid histidine kinase gene *CnNIK1*. Kiminori Shimizu, Antra Drivinya, Akira Yoshimi[1)], Chihiro Tanaka[1)], Susumu Kawamoto. Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Japan, [1)]Graduate School of Agriculture, Kyoto University, Japan.

Hybrid histidine kinases (HHKs) are known to be involved in drug resistance and osmosensitivity in filamentous fungi. We cloned a gene *CnNIK1* encoding an HHK from a human pathogen *Cryptococcus neoformans*. Comparison of genomic DNA and cDNA sequences revealed that the gene consists of 4149-bp open reading frame coding for 1383 amino acid residues interrupted by 13 introns, one of which is present in the 5• f untranslated region. Homology searches against GenBank database were carried out to identify conserved sequences such as HAMP domains, a phosphoacceptor histidine residue, an ATP binding domain and a response regulator domain, which are characteristics of HHK genes belonging to NIK1 family found in other filamentous fungi including *Neurospora crassa NIK1* and *Cochliobolus heterostrophus Dic1*. A *CnNIK1* disruption strain (delta *CnNIK1*) was created in order to study its biological function in *C. neoformans*. The MIC of delta *CnNIK1* strain against a fungicide fludioxionil was higher than 48 ug/ml, whereas that of wild type strain was 3 ug/ml, suggesting that *CnNIK1* is responsible for this drug sensitivity. However, delta *CnNIK1* strain did not show any growth defect in high osmotic conditions, which phenotype is seen in many fungal species lacking genes of NIK1 family, suggesting that the function of *CnNIK1* differs from those in other filamentous fungi. Further characterization of the strain is underway.

441. Genetic Regulation of Conidiation in *Trichoderma hamatum*. Johanna Steyaert[1], Margaret Carpenter[1], Alison Stewart[1], Travis Glare[2] & Hayley Ridgway[1]. [1]National Centre for Advanced Bio-Protection Technologies, PO Box 84, Lincoln University. [2]AgResearch Ltd., PO Box 60, Lincoln

Trichoderma spp. are ubiquitous soilborne ascomycetous fungi with superior biocontrol capabilities towards fungal phytopathogens. This capability has led to massive exploitation and currently Trichoderma spp. represent one third of all commercial fungal biocontrol agents sold globally. Commercial fungal biocontrol products involve bulk preparations of conidia, however considerable variability in conidiation rates exist between biocontrol agents. This variation can restrict the suitability of a particular strain for production. The majority of studies on Trichoderma conidiation have focused on the species T. viride and T. atroviride. These species form conidia in response to blue and near-UV light and/or nutrient deprivation. Conidiation proceeds in a highly co-ordinated fashion, however relatively little is known about the genetic basis of Trichoderma conidiation. In this study, conidiation in the lesser known biocontrol species T. hamatum is being investigated using a combined morphological and molecular approach. A selection of genes implicated in sporulation and the blue-light responses are currently being isolated and characterised from T. hamatum. Two genes, phr1 and cmp1, which were isolated previously from T. atroviride will be used as early and late markers of gene expression during the photoresponse in T. hamatum. Their expression will be used to define time points for harvesting comparable stage-specific RNA from T. hamatum and T. atroviride. Using degenerate PCR putative sporulation gene orthologues, rcoT, blr1 and blr2, have been identified in T. hamatum. Gene disruption studies on the orthologues are currently underway. Results of these studies, in addition to sequence and expression analysis, will be presented and discussed in relation to the current knowledge of the molecular basis of conidiation in Trichoderma and other filamentous fungi.

442. Regulation of gluconeogenesis in *Aspergillus nidulans*. Yumi Suzuki[1], Edyta Szewczyk[2], Meryl A. Davis[1] and Michael J. Hynes[1]. [1]Department of Genetics, University of Melbourne, Parkville, Victoria 3010, Australia. [2]Department of Molecular Genetics, Ohio State University, Columbus, OH 43210, USA.

Gluconeogenesis is required for the utilization of carbon sources metabolized via the TCA cycle. Gluconeogenesis requires the enzymes fructose-1,6-bisphosphatase and PEP-carboxykinase that mediate irreversible steps in glycolysis. There is at present little known about the mechanisms underlying the regulation of gluconeogenic genes in *A. nidulans*. The expression level of genes encoding these enzymes is significantly lower in strains with mutations of the *acuK* and *acuM* genes. These genes encode Zn(II)2Cys6 binuclear cluster proteins (Sealy-Lewis, personal communication) and are likely to be involved in transcriptional regulation of gluconeogenic genes. A double deletion strain of *acuK* and *acuM* shows a phenotype equivalent to that of the single deletion strains. It can therefore be concluded that these two proteins are involved in a single regulatory mechanism. Expression studies have been conducted on *maeA*, which encodes an NADP-dependent malic enzyme. Malic enzyme is required for growth on carbon sources metabolized via 2-oxoglutarate, such as proline. It converts malate to pyruvate, which is used to produce acetyl-CoA, and also provides NADPH. Mutations in *acuK* and *acuM* genes significantly reduce *maeA* expression supporting their role as regulatory proteins. However, the study showed that *maeA* had a different regulatory pattern from the gluconeogenic genes studied previously. One significant difference is that the high induction level brought about by proline is abolished in the presence of acetate. Acetate does not need to be utilized for this effect.

443. Differential gene expression during sclerotium formation in *Sclerotium rolfsii*. Johanna E. Takach and Scott E. Gold. Department of Plant Pathology, University of Georgia, Athens.

Sclerotium rolfsii, the causal agent of southern blight or white mold of peanut, survives poor environmental conditions by differentiating into sclerotia. Resistant sclerotia are critical to the persistence of disease potential in infested fields. Environmental factors, including variation in light, temperature, and oxygen are known to trigger sclerotium development, yet little is known about the genetic basis of this differentiation. To further understand this process, we have begun to identify and characterize genes up- and down-regulated during sclerotium formation. We are evaluating gene expression in a *S. rolfsii* isolate that consistently produces sclerotia in a highly programmed manner. With this strain we are using suppressive subtractive hybridization PCR (SSHP) to identify genes differentially expressed during sclerotium formation. Characterization of these genes will yield insight into the process and regulation of sclerotium differentiation. Preliminary results of this study will be presented.

444. Genetic and molecular basis of the dominant RIP suppressor of the Adiopopdoume strain of *Neurospora crassa*. Ranjan Tamuli and Durgadas P. Kasbekar. Centre for Cellular and Molecular Biology, Hyderabad 500 007, India

An assay for RIP was developed that makes use of a tagged duplication that targets RIP to the erg-3 gene. Using this assay seven dominant RIP suppressor strains of Neurospora were identified out of 446 strain screened. One of the seven suppressor strains is the Adiopodoume strain, which contains an active transposon, Tad. Interestingly, within this region I have identified a region that differs between mat A and mat a versions of the Oak Ridge background. I have also observed de novo DNA methylation, within this region. Understanding the molecular basis of dominant RIP suppression in Adiopopdoume might explain how Tad has survived in this strain.

445. Withdrawn
446. Withdrawn

447. Transcriptional control of dimorphic switching in *Penicillium marneffei*. Kaeling Tan and Alex Andrianopoulos. Department of Genetics, University of Melbourne, Melbourne, Victoria, 3010, Australia.

Many fungi are dimorphic; able to switch between yeast and filamentous form. Dimorphism is believed to be a crucial determinant for fungal pathogenicity in both plants and animals. *Penicillium marneffei* is an opportunistic pathogen of increasing medical importance due to the increase in immuno-compromised individuals especially in Southeast Asia. Previous studies demonstrated that there is differential expression of *aba*A in the different cell types of *P. marneffei*. Loss of *aba*A leads to asexual developmental abnormalities at 25ºC, similar to *A. nidulans*, such as the production of uninucleate, swollen chains of phialides (abacus structure) that fails to switch from aceropetal to basipetal division, and are unable conidiate. This implies that *aba*A may be involved in both yeast growths and in its conserved role in asexual development. The multi-nucleate yeast cells and arthroconidiating filamentous cells at 37ºC, compared to the uninucleate wildtype cells, suggests a role of AbaA in cell cycle control. These lead to the interest in investigating the regulation of *aba*A and its role in cell cycle regulation. The intimate coordination *abaA* expression involved in morphogenesis and cell cycle control is by studying the cyclin-dependent kinase (CDK) activity in *P. marneffei* (NimA and NimX^{Cdc2} homologue) during growth and development in the different cell types.
The *aba*A expression is investigated by assaying the reporter gene expression and by complementating the abaA promoter deletion strains to identify the promoter region required for AbaA regulation in yeast and asexual morphologies. DNA footprinting studies and mobility shift studies are performed in parallel to identify the specific regulatory sequences necessary for protein binding.

448. The multiple 3'-5' DNA helicases of *Neurospora crassa* cooperate in the *mei-3* -mediated homologous recombination repair. K. Suzuki, A. Kato, Y. Sakuraba , S. Tanaka, and H.Inoue* (Dept. Regulation-Biol., Fac. Sci., Saitama Univ., Saitama, Japan)

Homologous recombination repair and post-replication repair are important for restart of stalled and collapsed replication forks. The SRS2 gene of *Saccharomyces cerevisiae* encodes 3'-5' DNA helicase which functions in both of homologous recombination repair and post-replication repair. Here we identified the SRS2-homolog gene in the filamentous fungus *Neurospora crassa* and disrupted it by RIP. As the mutant showed significant sensitivity to several DNA-damaging agents, it was named mus-50. A series of epistasis analyses indicated that mus-50 belongs to the homologous recombination repair group, in which mei-3 (RAD51 homolog), mus-11 (RAD52 homolog), mus-48 (RAD55 homolog) and mus-49 (RAD57 homolog) are included, but mus-50 was not involved in post-replication repair. Also, double-repair deficient mutant carrying mus-25 (RAD54 homolog) and mus-50 mutations was not lethal, though combination of rad54 and srs2 mutations in *S.cerevisiae* is lethal. Tetrad analysis revealed that triple-repair deficient mutant carrying 3 mutations of mus-50 and two RecQ homologs, qde-3 and recQ2, is lethal. This lethality was suppressed by mei-3, mus-11 or mus-25 mutation. We also demonstrate that camptothecin-induced collapse of replication fork is repaired by two independent pathways: one is a QDE3- and MUS50-dependent pathway and another is a MUS25- and RECQ2-dependent pathway.

449. Promoter motifs needed for inducing *Phytophthora infestans* genes during zoosporogenesis in response to cold and increased membrane rigidity. Shuji Tani and Howard S. Judelson, Department of Plant Pathology, University of California, Riverside, CA 92521 USA

Zoospores, important components of the late blight disease caused by *Phytophthora infestans*, are released from sporangia in cold water (usually PinifC genes are regulated by a cold-induced inositol trisphosphate-mediated calcium signaling pathway. Using GUS reporter fusions, analyses of truncated, chimeric, and mutated *PinifC3* promoters revealed that a 7-bp sequence between positions -139 and -133 was sufficient for cold-induced transcription. This “cold box” was also detected in promoters of *PinifC1*, *PinifC2*, and orthologs from *P. sojae*. Protein(s) binding the cold box were detected by EMSA, and are being purified from nuclear extracts for sequence analysis. Furthermore, zoospore release and cold box-regulated transcription were induced by the membrane rigidifizer DMSO (mimicking a cold treatment), but inhibited by the membrane fluidizer benzyl alcohol. Our data delineate a cold signaling pathway in which sporangia perceive reduced temperatures via increased membrane rigidity, which triggers oscillations in cytosolic calcium, zoosporogenesis, and the induction of genes containing the cold box.

450. The ascospore discharge mechanism of *Gibberella zeae*. LUIS VELASQUEZ, Y. LeTourneau, C. Platt, H. Hallen and F. Trail. Department of Plant Biology, Michigan State University. East Lansing, MI. 48824

Gibberella zeae causes Fusarium headblight (FHB), one of the most destructive plant diseases in the world. The fungus overwinters in infested crop residues and is the main source of primary inoculum for next year's crop. Asci inside newly formed perithecia forcibly discharge sexual spores (ascospores) which are dispersed by wind and water to flowering hosts. The mechanism of spore discharge is the main focus of this research project. We have randomly mutated and subsequently screened mutants for a discharge minus colony. We have identified a mutated gene from a discharge minus colony that codes for a protein related to DNA binding. Additionally, we have characterized the ascus fluid discharged with the spores and determined that the major sugar component is mannitol. We have begun the targeted mutation of the genes involved in the mannitol biosynthesis of *G. zeae*. The two main enzymes in this pathway, Mannitol dehydrogenase (MtDH) and mannitol 1-phosphate dehydrogenase (M1PD) have been mutated. Additionally generation of a double mutant by mating is under way. Moreover, a series of candidate genes which could be involved in the ascospore discharge mechanism have been targeted for knockouts. We report, in this work, the progress of these approaches.

451. RPDA, a Class-i Histone Deacetylase, Is Essential for Viability of *Aspergillus nidulans*. Martin Tribus and Graessle Stefan. Innsbruck Medical University

During the past years it has become clear that chromatin represents an important regulatory element that affects nuclear processes such as DNA replication, recombination, DNA repair and transcription by tuning the accessibility of DNA for various regulatory factors. Cells have elaborated a specific machinery to modify nucleosomes for specific processes occuring in chromatin. Acetylation and methylation of the N-terminal tails of the core histones is the most prominent modification. Enzymes responsible for the dynamic equilibrium of histone acetylation are histone acetyltransferases (HATs) and histone deacetylases (HDACs). The latter form highly conserved protein among eukaryots. HDACs are categorized according to the yeast RPD3-like (class 1), HDA1-like (class 2), SIR2-like (class 3) and the plant specific HD2-like enzymes.
Recently, we have deleted representatives of each HDAC class in A. nidulans. Since we were not able to generate deletion mutants of RpdA, an RPD3-related class I HDAC, we put the rpdA coding sequence under the control of the inducible/repressible promoters alcA of A. nidulans and xylP of Penicillium chrysogenum. Repression of RpdA led to a drastic deficiency in viability of knock down strains. The data presented demonstrate that RpdA of A. nidulans is the first HDAC analyzed so far, which seems to be essential for survival of a eukaryotic organism.
This work was supported by the Austrian Science Foundation Grant FWF-P15439 to S.G.

452. Microarray analysis of the *Aspergillus niger* transcriptome reveals that XlnR plays an important role in the regulation of different pathways. Luisa Trindade and Leo de Graaff. Wageningen University, Fungal Genomics, Wageningen, The Netherlands

The XlnR protein was initially identified as a transcription activator of different genes encoding cellulolytic and xylanolytic enzymes in Aspergillus niger (van Peij et al., 1998). A number of experiments have been performed to elucidate the regulation mechanism of XlnR and the results suggest that the transcription of the xlnR gene is induced by the presence of D-xylose in the culturing media and repressed by D-glucose. As the mRNA levels of xlnR gene are very low, and they can hardly be detected by Northern blot analysis, further research is necessary to confirm these results. To study the XlnR regulon, XlnR mutants were grown under inducing (D-xylose and xylan), repressing (D-glucose) and neutral (sorbitol) culturing conditions. The whole transcriptome was examined by microarray analysis. The XlnR mutants used in these experiments are an xlnR knock out mutant, a mutant where XlnR is constitutively expressed, and the wild type phenotype. Comparison of the transcriptome of different XlnR strains under inducing and repressing conditions showed that XlnR regulates several genes that are involved in different pathways. Among these are genes encoding proteins involved in signal transduction, in the regulation of transcription, in sugar transport but also genes encoding enzymes.
Previous work of Graaff et al. (1994) suggested that the CreA transcription factor might play a role in the transcriptional regulation of the XlnR regulon. This has been shown for the enzyme encoding genes of the regulon, but whether CreA regulates the transcription of the xlnR gene still was uncertain. In order to investigate this the transcriptome of different single and double CreA derepressed and XlnR mutants were compared using both microarray and qPCR analysis. The results of these experiments lead to a new model for the transcription regulation of XlnR.
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Acknowledgments:
The authors thank DSM for giving us access to the Aspergillus niger genome and microarrays.

453. Transcriptional Profiling During Pathogenic Development of *Ustilago maydis*. M. Vranes, M. Scherer and J. Kaemper. Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany.

Little is known about the change of gene expression on planta and the role of the *b*-mating type during morphogenesis and pathogenicity of *Ustilago maydis* on planta. We have performed first experiments using DNA-Array analysis to address the gene expression in late tumour tissue. We identified 487 genes that were differentially expressed in planta when compared to haploid sporidia grown in liquid culture, among others genes encoding proteins with putative functions in plant cell wall degradation, iron uptake, lignin degradation and transport.
To address earlier stages of development we have employed a method to isolate fungal cells from the leaf surface. To identify genes differentially expressed on planta prior to plant penetration, we have performed DNA-Arrays with a solopathogenic strain containing an active bE/bW-heterodimer, mutant strains which are not able to penetrate and non-pathogenic wild type haploid sporidia. Our aim is to identify genes important for pathogenesis. Among those genes, which are upregulated in the solopathogenic strain, but are not expressed in the mutants, we have identified *riz1*, a gene encoding for a C_2H_2 zinc-finger-protein, as an important regulator of pathogenicity. Currently we are investigating the role of *riz1* during pathogenic development of *U. maydis*.

454. Rbf1 is a zinc finger transcription factor required for regulation of pathogenic development in *Ustilago maydis*. M. Scherer and J. Kaemper. Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., 35043 Marburg, Germany.

The phytopathogenic fungus *Ustilago maydis* has a dimorphic life cycle. For successful infection of its host plant maize two compatible haploid sporidia have to fuse on a leaf surface and form a filamentous dikaryon, which is able to penetrate the plant cuticula and subsequently leads to the fungal proliferation in planta. Pathogenic development is controlled by the multiallelic *b*-mating type locus encoding the homeodomain proteins bE and bW. bE and bW proteins expressed from different alleles can form a heterodimer, which is thought to regulate the b-dependent processes via its function as a transcriptional regulator. By expression profiling of b-mediated gene regulation we previously found 246 genes to be b-responsive.
We identified a gene encoding a C_2H_2 type zinc finger transcription factor as a very early induced b-target. Since deletion mutants are unable to form a b-dependent filament and to penetrate the plant surface, we named the gene *rbf1* (regulator of b-filament). Induced expression of *rbf1* leads to filamentous growth even in the absence of an active b-heterodimer. Microarray analysis revealed that Rbf1 is necessary as well as sufficient for the activation of a large subset of b-responsive genes. Thus, Rbf1 is an essential part of a regulatory cascade acting downstream of the b-heterodimer.
Current work is focussing on the identification of additional members of the regulatory cascade triggering pathogenic development.

455. Molecular aspects of dominant RIP suppression by large duplications and the associated barrenness in Neurospora. Meenal Vyas, Ravindran Chinnarajan and Durgadas P. Kasbekar. CCMB, Hyderabad, India

Previous studies in our lab have shown that large duplications can act as dominant suppressors of RIP in Neurospora possibly by titrating out the RIP machinery. Apart from this, seven wild isolated strains were identified as dominant suppressors of RIP in a screen in our lab. One of the suppressor strains, Sugar town, appears to harbor a large duplication based on its barren phenotype. We present data to map and molecularly characterize the Sugartown duplication and compare its size with that of synthetic large duplications like Dp (AR17), Dp (OY329) etc. that were shown to act as dominant RIP suppressors.
The role of RIP and RNAi – based mechanisms in rendering crosses involving duplication bearing strains barren, is also being studied in our lab. We report an intriguing observation where in crosses that are homozygous for Dp(AR17) and also defective for meiotic silencing and RIP, are fertile whereas a similar cross that is proficient for either RIP or meiotic silencing, is barren.

456. DNA methylation in the dimorphic fungus *Candida albicans*. Tithira Wimalasena, Heather Lee, Dolores Montiel, David Archer. School of Biology. University of Nottingham, University Park. Nottingham. NG7 2RD. UK.

Candida albicans is a dimorphic fungal pathogen of humans. It grows in yeast or mycelia forms depending on environmental conditions and, in the mycelial form, it is capable of invasive infection in humans. The dimorphic switch is associated with changes in gene expression patterns (Brown *et al.,* 1999) and some of these may be associated with DNA methylation. We know from chemical analysis of DNA extracted from *C. albicans* that there is low level DNA methylation and that the level is different in the two morphotypes (Russell *et al.,* 1987). We are investigating the presence of DNA methylation in *C. albicans* and its role in dimorphism. We have used methylation-sensitive restriction endonuclease isoschizomers (*HpaII, MspI and Dpn II, Sau3AI*) to analyse the methylation status of CCGG and GATC sequences. Amplified fragment length polymorphisms (AFLPs) were detected when DNA was digested with the isoschizomers suggesting that DNA methylation patterns are different in the yeast and mycelial forms. One of the AFLP bands has been cloned and sequenced and we will report on further analysis of methylation in that sequence. Furthermore, we have used the nucleotide analogue 5-azacytidine (Pancaldi *et al.,* 1988) to examine its impact on morphogenesis and to determine also the effect of azacytidine on DNA methylation using AFLP and Southern hybridisation analyses.
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457. Identification of Novel Genes Expressed During Conidiogenesis in *Beauveria bassiana* Using Suppression Subtractive Hybridisation. Jiang Wu[1], Hayley Ridgway[1], Drion Boucias[3] and Travis Glare[2]. [1]National Centre for Advanced Bio-Protection Technologies, PO Box 84, Lincoln University. [2]AgResearch Ltd, PO Box 60, Lincoln. [3]Department of Entomology and Nematology, University of Florida, Florida, U.S.A

The fungus Beauveria bassiana is an aggressive pathogen of a wide range of insect pests economically important to the agricultural industry. Although this fungus has considerable potential for development as a biopesticide, bulk production of the infective stage of the fungus (conidia) is inconsistent, restricting the choice of strain for commercial production. In this research we aim to enhance the development of B. bassiana fungal-based biopesticides by identifying and subsequently modifying the expression of sporulation genes to increase both consistency in in vitro spore production and resistance to the detrimental effects of UV light. Suppression subtractive hybridization (SSH) was used to isolate genes differentially expressed during conidiation of B. bassiana isolate B17. This was achieved by extracting RNA from both mature mycelium with conidia (tester mRNA population) and submerged synchronised mycelium without conidia (driver mRNA population). cDNA were synthesised for both the tester and driver populations. The tester and driver cDNAs were then hybridised and hybrid sequences removed, the remaining un-hybridised cDNA represent genes that are expressed during sporulation in the tester. The cDNAs were amplified with primary and secondary PCR and cloned into pGEM-T. A total of 91 clones were generated from transformation and PCR showed that most of clones contained 200-600 bp inserts. So far, twenty-three cDNA clone inserts have been sequenced and 17 fragments identified that were not redundant. The DNA sequence of each of the 17 cDNA fragments was used to search GenBank with both nucleotide and translated Blast searches and the sequences resulted in no notable similarity to sporulation genes from Aspergillus or Neurospora. Four of the cDNA fragments were used as probes for northern blot analysis. These fragments were strongly expressed in the lane containing RNA from the sporulating culture. Currently, the full DNA sequence of two novel genes are being isolated from a genomic library.

458. *Cryptococcus neoformans* senses CO2 during growth, mating, and infection. Bahn Yong-Sun and Joseph Heitman. Duke University

Carbon dioxide (CO_2) and bicarbonate (HCO_3^-) sensing and transport are essential cellular processes in diverse organisms from fungi to plants and mammals. The enzymatic interconversion between CO_2 and HCO_3^- is mediated by a ubiquitous protein, carbonic anhydrase (CA). In pathogenic fungi, including *Cryptococcus neoformans*, which can survive and proliferate in both environments and animal hosts, it will be crucial to understand the mechanisms of CO_2 sensing and its conversion to HCO_3^- since cells must accommodate dramatic changes in CO_2 concentrations during infection. Here we identified and characterized genes encoding CA in *C. neoformans* to investigate the role of CO_2 sensing in the human pathogenic fungus. *C. neoformans* contains two ?-CA proteins, Can1 and Can2, which are homologous to CA in other fungi but distinct from ?-CA in mammals. Can2, but not Can1, was found to be required for normal growth in ambient conditions. The can2? mutant was able to resume growth in the presence of high CO_2 concentrations (5%). Both Can1 and Can2 are dispensable for capsule and melanin production and stress responses with or without high CO_2 pressure. Most interestingly, we found that CO_2 completely blocks mating between wild-type *MAT*? and **a** strains by inhibiting cell fusion, but not filamentation. This CO_2-dependent inhibition of mating is reversed by can2, but not by can1 mutations. Regardless of their growth defect in ambient conditions, can2? mutants were found to survive better than the wild-type in the rabbit model of systemic cryptococcosis. In conclusion, the carbonic anhydrase Can2 catalyzes CO_2-HCO_3^- conversion that modulates in vitro growth, mating, and virulence of *C. neoformans*

Developmental Biology

459. The cyclin Cln3p links G1 progression to hyphal and pseudohyphal development in *Candida albicans*. Catherine Bachewich and Malcolm Whiteway. BRI, National Research Council of Canada

G1 cyclins coordinate environmental conditions with growth and differentiation in many organisms. In the pathogen *Candida albicans*, differentiation of hyphae is induced by environmental cues, and was thought to occur independent of the cell cycle. Repressing the G1 cyclin Cln3p under yeast growth conditions caused yeast cells to arrest in G1, increase in size, and then develop into hyphae and pseudohyphae, which subsequently resumed the cell cycle. Differentiation was dependent on Efg1p, Cph1p and Ras1p, but absence of Ras1p was also synthetically lethal with repression of *CLN3*. In contrast, repressing *CLN3* in environment-induced hyphae did not inhibit growth or the cell cycle, suggesting that yeast and hyphal cell cycles may be regulated differently. However, hyphal compartments were longer and morphogenesis was not normal in *CLN3*-repressed hyphae, suggesting that cell cycle progression and hyphal growth may be co-regulated. We are currently determining the transcription profiles of *C. albicans* cells depleted of *CLN3* to screen for potential factors that mediate the developmental response. Therefore, absence of a G1 cyclin activates developmental pathways in *C. albicans* and uncouples differentiation from normal environmental controls. Since blocking the cell cycle at other stages produces different responses, the data suggest that G1 phase of the cell cycle may play a critical role in regulating hyphal and pseudohyphal development in *C. albicans*.

460. Identification of Genes Differentially Expressed Early in the Sexual Development of *Schizophyllum commune*. Kirk A Bartholomew. Sacred Heart University, Fairfield, CT, USA

The well characterized products of the complex A and B mating-type loci of *Schizophyllum commune* control activation of sexual development when monokaryotic mycelia of non-self mating type interact. Both the products of the A loci (interacting homeodomain proteins) and the B loci (lipopeptide pheromones and associated G-protein coupled receptors) are proposed to initiate the events of sexual development by controlling the differential expression of target genes in mating mycelia eventually leading to the formation of fully dikaryotic mycelia with binucleate cells and associated clamp connections. In this ongoing project modifications of a membrane culture technique were used to mate colonies of sexually compatible monokaryons by transferring them into close proximity. RNA isolated from the mated mycelia prior to the fully dikaryotic stage of sexual development was compared with RNA from unmated mycelia from the same sexually compatible monokaryons by the reverse transciptase differential display polymerase chain reaction method (RTDDPCR) in order to identify genes differentially expressed during the early stages of sexual development. RTDDPCR analysis with 48 primer pairs identified 15 partial cDNAs that were strong candidates for differential expression. Progress in cloning, sequencing and confirming the differential expression of the candidate cDNAs will be reported.

461. Peroxisomes and sexual differentiation in *Podospora anserina*: characterization of the peroxisomal protein receptors PEX5 and PEX7. C. Bonnet, Espagne E., Zickler D. and Berteaux-Lecellier V. Institut de Génétique et Microbiologie, UMR8621, Université Paris sud, Bât 400, 91405 Orsay Cedex, France.

Peroxisomes are highly adaptive organelles involved in numerous enzymatic functions and in various developmental decisions. In *P. anserina* we have shown that they are required at a specific step of the sexual cycle. The peroxisome biogenesis *pex2* mutant (Zellweger syndrome in Human) is unable to switch from the mitotic to the meiotic state.
To determine more precisely the link between peroxisomes and differentiation, we characterized the two peroxisomal receptors PEX5 and PEX7. While very few peroxisomal matrix proteins possess the peroxisomal targeting signal recognized by PEX7, PEX5 is responsible for the import of most peroxisomal matrix proteins. The *P. anserina pex5* gene encodes a protein of 665 aa composed of 7 TPR repeats while *pex7* encodes a protein of 358 aa with 6 WD40 repeats. These two proteins share 90% and 60% similarity with their orthologs in *Neurospora crassa* and Human, respectively.
Contrary to *pex2* mutants, both *delpex5* and *delpex7* deleted strains are able to achieve the sexual cycle. However, a delay is observed in the transition from the mitotic to the meiotic stage in the *delpex5* homozygous crosses. Analysis of the double mutant *delpex5delpex7* is under investigation. The properties and cytological characterisation of the known *pex* mutants will be discussed in the frame of the involvement of peroxisomes in sexual development.

462. Does RIP occur at a specific stage of premeiotic development in *Podospora anserina*? Khaled Bouhouche, Sylvie Arnaise, and Robert Debuchy. Institut de Genetique et Microbiologie, UMR8621, Orsay, France.

RIP is a homology-dependent gene-mutation mechanism which takes place inside the perithecia before premeiotic replication. Whether RIP occurs during a specific stage of the complex perithecial development preceding the premeiotic replication remains unknown. These stages are investigated in details in *Podospora anserina*. In this heterothallic fungus, fertilization is followed by the formation of plurinucleate cell containing nuclei of opposite mating type which recognize each other to form dikaryotic hyphae. The tip cell of dikaryotic hyphae differentiates into a specialized cell where karyogamy and meiosis take place. Previous analyses of the four mating-type genes demonstrate that three of them control the internuclear recognition (IR) and that the fourth is essential for the development of dikaryotic hyphae. To determine if RIP occurs before IR, or between IR and dikaryotic hypha formation, or during dikaryotic hypha development, we constructed mating-type gene duplication at the mating-type locus and searched if RIP resulted in an altered progeny. If a gene is RIPed at a stage preceding the step under its control, the altered gene would lead to a mutant phenotype. In contrast, if RIP occurs later than the step controled by the duplicated gene, the strain would not display any defect in crosses, but defective crossing of the progeny containing the RIPed gene would reveal the mutation. Preliminary results with the duplication of a mating-type gene involved in IR will be discussed in the poster.

463. Deletion analysis of genes regulated by cAMP-dependent protein kinase A in *Ustilago maydis*. Kylie J. Boyce, Luis Larraya, Howard Chang, Cletus D'Souza and James W. Kronstad. The Michael Smith Laboratories, University of British Columbia, Vancouver, B.C. Canada V6T 1Z4

In the corn smut fungus *Ustilago maydis*, development and virulence are regulated by a cAMP-dependent protein kinase A (PKA) pathway and at least one mitogen-activated protein kinase (MAPK) pathway. To identify additional genes regulating development and virulence in *U. maydis* we have used serial analysis of gene expression (SAGE) to analyze the transcriptomes of wild-type and mutant strains defective in either the catalytic (*adr1*) or regulatory (*ubc1*) subunit of PKA. Mutation of the catalytic unit of PKA results in filamentous cell growth, whereas mutation of the regulatory PKA subunit results in a multi-budded phenotype. Using SAGE we have identified numerous genes whose transcript abundance is altered by a mutation in PKA and is hence up or down regulated during filamentation or budding. In this study we describe the deletion of some of these genes with a focus on the deletion of a gene encoding a Cdc11p, septin homolog. Deletion of the *U. maydis* Cdc11p homolog, *sep1*, results in cells with division defects including mis-localization of chitin and failure of cells to separate, as well as morphological and nuclear defects. Strains with the *sep1* mutation fail to grow filamentously in response to fatty acids, despite being able to form filaments during mating. Deletion of *sep1* does not inhibit pathogenicity but results in cells types with altered morphology *in planta*. The generation of *ubc1 delta sep1* strains has showed that deletion of *sep1* prevents the multi-budded phenotype of the *ubc1* strain.

464. Analysis of the regulatory multprotein complex COP9 Signalosome in *Aspergillus nidulans*. Silke Busch, K. Helmstaedt, O. Bayram, O. Valerius, Gerhard H. Braus. Institut für Mikrobiologie und Genetik, Georg-August-Universitaet, Grisebachstrasse 8, D-37077 Goettingen (GER)

The COP9 signalosome (CSN) is a eukaryotic multiprotein complex that connects various signals with multiple downstream molecular pathways controlling cellular differentiation and development. In higher eukaryotes, disfunction of the CSN is embryonic lethal, whereas in *Aspergillus nidulans* deletion of CSN subunits leads to viable strains with severe developmental defects. We want to understand the molecular networks controlled by CSN action in *A. nidulans* as a genetically amaneable eukaryotic model system.
In higher eukaryotes, the CSN is composed of eight conserved subunits. We identified proposed open reading frames for all of them in the *A. nidulans* genome sequence. Using the tandem affinity purification method (TAP) we purified a complex containing several CSN subunits as proven by mass spectrometry. We are currently improving the method to additionally identify CSN associated proteins.
Function of the CSN involves 26S proteasome-dependent regulation of cellular protein levels, mainly by the signalosome's intrinsic deneddylation and associated kinase activities. Accordingly, we observed a different protein expression pattern in a wild-type compared to a *csn* deletion strain in 2D gel analyses. Differentially expressed proteins were identified by mass spectrometry. These potential downstrem targets of CSN action hint at a CSN function in regulation of metabolism, cytoskeleton composition and stress response.

465. Negative regulation of sexual development by specific interactions of IndB/D proteins with NsdD in *Aspergillus nidulans*. Nak-Jung Kwon, [1]Dong-Min Han, and Suhn-Kee Chae, BioMed RRC and Dept. of Biochem., Paichai University, Daejeon 302-735 Korea, and [1]Division of Biological Science, Wonkwang University, Iksan 570-749, Korea.

A GATA type transcription factor NsdD has been known as a positive regulator of sexual development in *A. nidulans*. Previously, we isolated two novel proteins, IndB and IndD, that interacted with NsdD by the yeast two-hybrid screening. *In vitro* and *in vivo* associations of NsdD with IndB and IndD have been confirmed. Mutated NsdD proteins carrying change in the zinc-finger motif failed to interact with IndB/D, indicating that the intact zinc-finger domain is required for the interactions. Other GATA type proteins of LreA, LreB, and AreA failed to interact with IndB/D, suggesting that NsdD-IndB and -IndD interactions are specific. Northern analysis showed that both of the indB and indD transcripts were highly elevated in *veA* disruptants, indicating that VeA represses the *indB/D* gene expression. Overexpression of *indB* and/or *indD* strongly inhibited sexual development in veA+ backgrounds. We also identified that IndB/D proteins inhibited DNA bindings of NsdD at putative GATA sites *in vitro*. Therefore, our results suggest that indB/D function on sexual differentiation negatively via binding to the zinc-finger domain of NsdD to interfere NsdD function, and are regulated by the *veA* gene, providing a clue of modulating process in sexual development promoted by NsdD and VeA. [Supported by grants from KOSEF]

466. Sumoylation is required for differentiation and DNA repair in *Aspergillus nidulans.* Nak-Jung Kwon, Jeong-Hwa Park, and Suhn-Kee Chae. BioMed RRC and Division of Life Science, Paichai University, Daejeon 302-735, Korea

SUMO modifies functions of proteins in various ways when attached covalently. The first step of sumoylation is the formation of a thiol ester linkage between the C-terminal glycine of SUMO and an active site cysteine of an SUMO activating enzyme complex, Uba2 and Aos1 (E1). The second step involves the transfer of SUMO to a cysteine in the active site of an SUMO conjugating enzyme of Ubc9 (E2). In the last step, the E2 enzyme may cooperate with an protein ligase (E3) to form an isopeptide bond between the C-terminal glycine of SUMO and a lysine-amino group in the target. Enzymes involved in the sumoylation process are well conserved in *A. nidulans* based on the analysis of *A. nidulans* genomic DNA sequence database. The *sumO* and *ubcN* genes of A. nidulans encoding homologs of SUMO and Ubc9, respectively, were cloned. Null mutants of *sumO* and *ubcN* were not lethal in *A. nidulans*. Mycelial growth rate was not much affected but conidiation hardly occurred in both null mutants. Furthermore, cleistothecium was never found in various growth conditions, but Hulle cells were still observed. The *sumO* transcript was expressed to the similar level during asexual and sexual differentiation. *delta-sumO* and *delta-UbcN* exhibited high sensitivities to 4-NQO, MMS, CPT, and HU compared to those for wild-type. In conclusion, sumoylation process is required for proper differentiation and DNA repair in *A. nidulans*. [Supported by KOSEF]

467. Functional study of *SMR1*, a mating-type gene which does not control self-non self recognition in *Podospora anserina*. Evelyne Coppin, Sylvie Arnaise, Khaled Bouhouche, Xavier Robellet, and Robert Debuchy. Institut de Genetique et Microbiologie, UMR 8621 CNRS-Universite Paris Sud, Orsay, France

The sexual reproduction of *P. anserina* requires the coordinate action of 4 mating-type genes. Three of them encode transcriptional factors, which control recognition between *mat+* and *mat-* sexually compatible cells during fertilization, and between nuclei after fertilization, at the transition from a plurinucleate to a dikaryotic stage when ascogenous hyphae are formed. The fourth gene, *SMR1*, is involved in pre-fertilization or initial steps of perithecial development, and is essential for the dikaryotic stage. By contrast, it is dispensable for the development of uniparental ascogenous hyphae resulting from *mat* gene mutation affecting nuclear pairing. Orthologs of SMR1 are present in all Sordariomycetes analyzed to date, but the molecular function of these genes remains unknown. Subcellular localization of SMR1 by GFP tagging suggests that it has a cytosolic localization, which does not support previous prediction that it is a transcription factor. A *SMR1* disruption results in barren perithecia and suppressors restoring fertility were previously isolated. All of them affect *mat* genes and nuclear pairing. We have constructed 30 new alleles by alanine scanning and found one mutation which leads to sterility in cross. This point mutation is used to screen suppressors with the aim of characterizing new genetic interactions helping to understand the role of SMR1.

468. The function of the coding sequences for the putative pheromone precursors in *Podospora anserina* is restricted to fertilization. Evelyne Coppin, Christelle de Renty, and Robert Debuchy. Institut de Genetique et Microbiologie, UMR 8621 CNRS-Universite Paris-Sud, Orsay, France

We addressed the role of the pheromone precursor genes in *P. anserina*, and explored regulation of their expression by *mat+* and *mat-* mating types. We cloned these genes using their previously isolated homologs in *Neurospora crassa* and *Cryphonectria parasitica*. They were named *mfp* and *mfm* (mating factor plus and minus) since their transcription is controlled by *mat+* and *mat-*, respectively. The *mfp* gene encodes a 24-aa peptide ending with the CAAX motif, characteristic of fungal lipopeptide pheromone precursors. The *mfm* gene encodes a 221-aa polypeptide, which contains two 13-residue repeats assumed to correspond to the mature pheromone. Deletion of each coding sequence impaired male fertility in a mating-type specific manner, without affecting female fertility and vegetative growth. We managed to cross null mutants lacking both *mfp* and *mfm*, by complementation or transient expression of the required pheromone gene to trigger fertilization. These crosses were fertile, showing that once fertilization had occured, the pheromones are no longer necessary for the completion of the sexual cycle. Finally, we subtracted both genes from mating-type control by replacing their 5 UTR by the constitutive glyceraldehyde-3-phosphate dehydrogenase promoter and found that expression of both constructs is repressed post-transcriptionally by the non-cognate mating type.

469. Ribonucleoprotein particles that shuttle along microtubules are essential for pathogenic development in *Ustilago maydis*. Philip Becht, Julian König, Janine Koepke, and Michael Feldbrügge. Max-Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Karl-von-Frisch-Strasse, 35043 Marburg, Germany

RNA-binding proteins function as developmental regulators in eukaryotes. To test whether this holds true for pathogenic development of *Ustilago maydis*, the causative agent of corn smut, we used a reverse genetic approach. We deleted 18 open reading frames encoding putative RNA-binding proteins. One of these designated Rrm4 contained three N terminal RNA recognition motives with an architecture known from ELAV proteins (embryonic lethal abnormal vision). The corresponding deletion strain was impaired in filamentation and pathogenicity. Using time-lapse fluorescence microscopy we were able to demonstrate that Rrm4 assembled into particles that shuttled along microtubules. Thus, Rrm4 is apparently part of ribonucleoprotein complexes that transports RNA from the nucleus to the cell poles. Currently, we are focusing on unravelling the molecular composition of the Rrm4-containing particles to identify the cargo and the mode of transport.

470. Few sequence constraints hinder *Schizophyllum* pheromone function in yeast. Thomas Fowler, Joseph Pare, and Christopher Baker, Dept of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405 USA
G protein-coupled pheromone receptors of *Schizophyllum commune* can distinguish among the many dozens of mating pheromones in the species. One group of highly similar lipopeptide pheromones were examined for clues to their activity and specificity using extensive mutagenesis at several amino acid positions within the mature pheromones. An heterologous assay was utilized in which *S. commune* pheromones and pheromone receptors were functionally expressed in the yeast *Saccharomyces cerevisiae*. Successful mating of these transgenic yeast strains identified functional mutant pheromones. Many amino acid positions in the mature pheromones are much more mutable than might be predicted from sequence comparisons. Pheromone Bbp2(4) tolerates at least 17 different amino acid substitutions at one amino acid position while more than 10 changes are tolerated at other positions with little effect on specificity. We have begun to test some of the mutant pheromones directly in *S. commune* to explore the reason for amino acid conservation of the mature pheromones in *S. commune* in contrast to the results of the heterologous assay. One possibility is that *S. commune* requires these conserved residues for pheromone processing and export, but not for receptor activation, while yeast employs a very flexible processing and export mechanism for these heterologous pheromones. Regardless of possible differences in pheromone processing and export, the features within the amino acid sequences of mature pheromones that are required for receptor activation are surprisingly minimal.

471. Exploring appressorial formation in the cereal biotroph, powdery mildew. Sally Gilbert[1], Andy Bailey[2], Bart Fraaije[1], Hans Cools[1] and John Lucas[1]. [1]Plant-Pathogen Interactions Department, Rothamsted Research, Harpenden, Herts AL5 2JQ England [2]School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG England.

Powdery mildew (*Blumeria graminis*) is an obligate, fungal biotroph which invades its host using an infection structure, termed appressorium. There is considerable interest in understanding the sequence of events that leads to appressorial formation and ultimately successful penetration of the host plant. A greater understanding of the signal transduction pathway has and continues to facilitate the development of novel fungicides that target specific stages in this pathway.
The main aim of this project is to use real-time RT-PCR to identify potential target-encoding gene(s) of a novel fungicide known to inhibit appressorial formation. Six genes that play a role in appressorium formation were chosen. These encode an integral membrane protein (*PTH11*); a G protein alpha subunit (*Bgene*); a Mitogen-activated Protein kinase (*MAP1*); a Protein Kinase C (*PKC*); a cAMP-dependent Protein Kinase A (*CPKA*); and an Adenylate Cyclase (*BAC1*). In addition, a Catalase/Peroxidase encoding gene (*CPX*) was also chosen as it is involved in scavenging activated oxygen species generated during the plant/pathogen interaction. TaqMan® probes have been designed and used to determine changes in expression of these genes in the presence of fungicides and/or inhibitors as compared to the untreated control.

472. Characterization of components of an apoptotic machinery in the filamentous ascomycete *Podospora anserina*. Andrea Hamann, Heinz D. Osiewacz. Botanisches Institut, J.W. Goethe-Universitaet, Marie-Curie-Str. 9, D-60439 Frankfurt, Germany

During the last years several analyses demonstrate the existence of apoptotic processes in the ascomycete fungi *Saccharomyces cerevisiae* and two *Aspergillus* species. The search for homologues in the genomic sequence of *P. anserina* (http://www.genoscope.cns.fr/externe/English/Projets/ Projet_GA/GA.html) resulted in the identification of a number of putative members of the apoptotic machinery including two metacaspases, one apoptosis-inducing factor (AIF), and one AIF-homologous mitochondrion-associated inducer of death (AMID) and others. Here we present first data on the characterization of selected components via over-expression and/or deletion of selected genes. During the course of investigations we optimized the gene replacement procedure developed by Chaveroche et al. (2000, Nucleic Acids Res. 28: E97) for use with *P. anserina*. The features of this modified method are reported. Utilizing this strategy, we generated and initially characterized several strains in which different genes encoding putative components of the apoptotic machinery were disrupted. The deletion of *PaMca1*, a gene encoding a homologue of a yeast metacaspase, resulted in increased life span and enhanced stress tolerance. The influence of apoptotic components on stress tolerance, life span and on spore degeneration in crosses between so-called killer and non-killer strains will be discussed.

473. The *Aspergillus nidulans silG* gene functions in repression of sexual development in response to light. Kap Hoon Han[1], Bang-Yong Lee, and Dong-Min Han. Div. of Biological Science, Wonkwang University, Korea [1]Dept. of Plant Pathology, Seoul National University, Korea

The *Aspergillus nidulans nsdD* gene encodes a GATA-type transcription factor that is required for sexual development. To further understand the NsdD-mediated regulatory cascade, we isolated various suppressors of *nsdD* (SND). The *silG* gene presented in here was identified as a multi-copy suppressor of one of the *snd* mutations. Multi-copy of *silG* blocked cleistothecia development in an SND mutant. The *silG* gene is predicted to encode a 703 aa polypeptide with three C2H2 zinc finger DNA-binding domains at the C-terminus. The *silG* null mutant produced a high number of cleistothecia even under the visible light, which normally inhibits sexual development. However, high osmolarlity or poor carbon sources blocked sexual development of the *silG* deletion mutant, suggesting that SilG may play a specific role in negative regulation of sexual development in response to light. Further supporting this hypothesis, over-expression of *silG* resulted in a great reduction of sexual development in dark, which preferentially enhance sexual development in wild type. Accumulation of *silG* mRNA undulated throughout the lifecycle in a certain recurring pattern. Interestingly, *silG* mRNA levels elevated upon exposure to light, and this response requires the functional *A. nidulans wcA*(*lreA*) gene. Moreover, mRNA level of *silG* in *veA1* mutant was increased in dark, implying that *silG* functions downstream of *veA* and VeA may repress *silG* expression in dark, which leads to sexual development.

474. Oxidative stress and cell differentiation in *Neurospora crassa*. Wilhelm Hansberg, Leonardo Peraza, Jesús Aguirre, Karen Delfín, Nallely Cano. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México D. F., México.

Morphogenetic transitions of *N. crassa* life cycles are considered responses to a hyperoxidant state. If so, mutant strains in antioxidant enzymes should favor and in pro-oxidant enzymes should inhibit development. A catalase-3 (*cat–3*) null mutant strain showed increased protein oxidation and carotene levels in the dark, indicating oxidative stress. The strain formed hyphae aggregates and produced more aerial hyphae and conidia than the WT (1). A catalase-peroxidase (*cat–2*) null mutant strain was sensitive to peroxides, formed more arthroconidia and more protoperithesia and perithesia than the WT. In contrast, NADPH oxidase-1 (*nox–1*) null mutant strain formed less aerial hyphae and no protoperithesia and a *?nox–2* formed ascospores that could not germinate. These results indicate that oxidative stress is required for cell differentiation in *N. crassa*.
1) Michán S, Lledías F, Hansberg W (2003) Asexual development is increased in *Neurospora crassa catalase-3* null mutant strains. Eukaryotic Cell 2:798-808.
Acknowledgements: CONACyT C01-40697, DGAPA/UNAM IN228405

475. Functional Comparison of Cdc42 and Rac1 in the Dimorphic Fungus *Ustilago maydis*. Andrea Hlubek, Kay Schink and Michael Bölker. Fachbereich Biologie, Universität Marburg, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany

Cdc42 and Rac1 are small GTPases which regulate various cellular processes like morphogenesis, vesicle trafficking, cytokinesis and cell polarity. Guanine nucleotide exchange factors (GEFs) convert the GTPases to their active, GTPbound form; GTPase activating proteins (GAPs) stimulate the GTPase activity.
Both genes are not essential for viability in the dimorphic fungus *U. maydis*, but they share at least one important function. We could show that *U. maydis* Cdc42, but not Rac1, complements the lethal phenotype of the temperature sensitive *cdc42-1* allele in *Saccharomyces cerevisiae*. To define the regions that are responsible for the specificity of these proteins, we generated a set of chimeric proteins, which were checked for complementation of both *U. maydis* and *S. cerevisiae* mutants. These experiments demonstrate that the region between amino acids 41 and 56 is necessary and sufficient to determine the specificity of the GTPases. This could be due either to specific activation of the GTPases by their cognate GEFs or to their interaction with specific downstream effectors. To distinguish between these possibilities we are currently characterizing further GEFs specific for either Cdc42 or Rac1. Preliminary indications suggest that a single amino acid might be important for specific interactions with certain GEFs. Corresponding chimeras of human Cdc42 and Rac1 are currently tested by transient expression in mouse fibroblasts.

476. A putative sugar transporter is involved in the regulation of mushroom development in the basidiomycete *Schizophyllum commune*. Peter Sage, Josh White, Alaap Shah, Gail Palmer and Stephen Horton. Department of Biological Sciences, Union College, Schenectady, NY 12308 USA

Mushroom development in basidiomycetes is regulated by both genetic and environmental factors. Nutrient depletion is one of the necessary prerequisites for fruiting in these fungi. The gene *sts1* encoding a putative sugar transporter has been isolated from *Schizophyllum commune* by means of a two-hybrid screen using a bait protein implicated in mushroom development. The predicted protein is 550 amino acids in length, and has 12 transmembrane domains typical of these transporters. Co-transformation of a dominant activated mutant version of *sts1* into a homokaryotic fruiting recipient strain drastically inhibits mushroom formation in a small number of transformants. Overexpression of this mutant gene using the highly expressed *sc3* promoter resulted in a much greater number of affected transformants. Immunofluoresence studies using anti-myosin antibodies showed an accumulation of staining at many of the hyphal septa in non-fruiting *sts1* overexpression strains. We hypothesize that the mechanism of the block in fruiting observed in the mutant *sts1* overexpression strains may involve disruption of myosin-mediated vesicle transport. Grant support from the NIH-AREA program is gratefully acknowledged.

477. Control of the circadian clock in *Aspergillus flavus* by LreA and LreB. L. R. Milde[1], A. Greene[2], D. Bell-Pedersen[2], N. P. Keller[1]. [1]Department of Plant Pathology, University of Wisconsin, Madison, WI 53706; [2]Program in Biological Clocks, Department of Biology, Texas A&M University, College Station, TX 77843

Endogenous circadian clocks generate and control daily rhythms in biochemical, physiological, and behavioral processes. Circadian rhythms are widespread in nature, occurring in organisms ranging from cyanobacteria to humans. In *Aspergillus flavus*, fungal clock mechanisms have been shown to control the production of sclerotia, which are important to the development and survival of the fungus. Putative orthologs of *Neurospora crassa* clock components, WC-1 and WC-2, have been identified in the Aspergilli and are called LreA and LreB, respectively. Using RNA interference technology, both genes were simultaneously silenced in *A. flavus*, yielding transformants aberrant in rhythmic sclerotial production. Further examination will help to distinguish the specific functions of LreA and LreB in the Aspergilli and to gain a better understanding of circadian-control of the survival structures and virulence determinants of the pathogenic Aspergilli.

478. Role of a MAP kinase cascade in the development of an epigenetic cell degeneration. Sébastien Kicka, Fabienne Malagnac, Andrew K. Sobering, Crystel Bonnet, Latha Prabha Ganesan and Philippe Silar. Institut de Génétique et Microbiologie, Université de Paris Sud, 91405 Orsay cedex France

PaASK1, a MAPKKK, and PaNox1, a NADPH oxidase are essential to develop Crippled Growth (CG), an epigenetic cell degeneration of the filamentous fungus *Podospora anserina*. A model based on the bistable and hysteretic behaviour of the PaASK1 MAP kinase cascade has been proposed to explain the properties of CG, especially its triggering by *C*, a cytoplasmic and infectious factor. In this model, *C* is the active form of the cascade. To confirm this model, the downstream MAPKK and MAPK genes have been cloned and null alleles recovered. Inactivation of the MAPKK and MAPK genes triggers the same phenotypes as the inactivation of the MAPKKK gene, confirming the crucial role of the MAP kinase module in the development of CG. Strains harbouring transgenes expressing a MAPK-HA fusion protein have been constructed and used to evaluate the phosphorylation status of the MAPK during Normal and Crippled Growth. Data show that phosphorylation of the MAP kinase perfectly correlates with the presence of the *C*, as expected from the model. In addition, lack of phosphorylation of the MAP kinase in the *PaNox1* mutant confirm that PaNox1 acts upstream of PaASK1. These data are the first steps towards the deciphering of the molecular mechanisms involved in the generation of the Crippled Growth cell degeneration.

479. The *nsdC* gene encoding a novel C2H2-type zinc finger protein is a key activator of sexual development in *Aspergillus nidulans*. Hye-Ryoun Kim, Kap Hoon Han[1] and Dong-Min Han. Division of Life Science, Wonkwang University, Korea [1]Seoul National University, Korea

Sexual development in *Aspergillus nidulans* is affected by a number of genetic and environmental factors. Previously, we isolated various NSD (never in sexual development) mutants and identified a gene called *nsdD* encoding a GATA-type transcription factor necessary for sexual development. Here, we report the identification and characterization of the *nsdC* gene, which is predicted to encode a 643 aa polypeptide with a novel C2H2-type zinc finger DNA binding domain. While deletion of *nsdC* resulted in the typical NSD phenotype, i.e., lack of sexual development, overexpression of *nsdC* not only enhanced formation of sexual fruiting bodies (cleistothecia) but also overcame inhibitory effects of certain stresses on cleistothecial development, indicating that NsdC is a key positive regulator of sexual development. The *nsdC* gene generates two distinct transcripts, 3.2 and 2.8 kb, and its 5' UTR is ~1.5 kb with two relatively long introns (168 bp and 212 bp). Differential accumulation of two *nsdC* transcripts in various stages of growth and development as well as under the varying environmental conditions indicates that *nsdC* is subject to complex transcriptional regulation. Carbon source dependent retarded vegetative growth and hyper-active asexual development phenotypes were observed in the *nsdC* deletion mutant. This result further supports the idea that balanced progression of sexual and asexual development is necessary for the maintenance of genetically programmed lifecycle.

480. A homeobox-domain protein represses sexual development in *Aspergillus nidulans*. Jee-Hyun Kim, Dong-Beom Lee and Dong-Min Han. Division of Biological Science, Wonkwang University, Korea

Previously we identified *NsdD* , a crucial activator for sexual development in Aspergillus nidulans. While increased dose of *nsdD* caused elevated formation of fruiting bodies (cleistothecia) even under the unfavorable environmental conditions, *nsdD* mRNA levels did not change, suggesting that multiple copies of the *nsdD* promoter might relieve repressive effects by negative regulators. To dissect complex regulatory networks of sexual development, we identified a multi-copy suppressor (*nrsA*) that abolished enhanced cleistothecia formation by increased dose of nsdD. The *nrsA* gene is predicted to encode a novel 293 aa protein with a homeobox domain at the C terminus. Deletion of *nrsA* resulted in elevated cleistothecial development even in the presence of stresses, but caused lowered asexual sporulation. Conversely, forced expression of *nrsA* blocked formation of cleistothecia, while enhancing asexual development, indicating that *nrsA* act as a key negative regulator of sexual development, which may confer properly balanced progression of sexual and asexual development in response to environmental factors. Steady state mRNA levels of *nrsA* increased at the later phases of sexual/asexual development as well as in the presence of osmotic stresses during vegetative growth. Taken together, we propose that the homeo-domain protein NrsA administrates coordinated regulation of two distinct developmental programs in *A. nidulans*.

481. A hydrophobin gene, *VDH1*, is involved in microsclerotial morphogenesis and spore viability in *Verticillium dahliae* (Kleb). A. Klimes[1] and K.F. Dobinson[2]. [1,2]Department of Biology, University of Western Ontario, London ON N6A 5C1, Canada; [2]Southern Crop Protection and Food Research Centre, Agriculture and Agri-food Canada, 1391 Sandford Street, London, ON N5V 4T3, Canada.

The plant pathogen *Verticillium dahliae* (Kleb) is characterized by the production of microsclerotia (MCS), the desiccation and cold tolerant resting structures that serve as a primary source of disease inoculum in the field. Despite the critical role of these structures in the fungus's life cycle, the molecular mechanisms of microsclerotial development are not well understood. We have identified a class II hydrophobin gene, *VDH1*, that is important to the formation of these critical structures. A green fluorescent reporter gene under the control of the wild type *VDH1* promoter is highly expressed in developing MSC, conidiophores and spores. Targeted disruption of *VDH1* severely inhibits MCS production. Although *vdh1*, mutants do produce conidiophores and spores, the spores are less tolerant to desiccation than are wild type spores. *vdh1* disruption mutants are able to cause disease in tomato plants, indicating that the gene is not needed for pathogenicity. While its importance in the development of *V. dahliae* resting structures is apparent, the precise role of *VDH1* in morphogenesis is not yet clear. We are using microarray analysis to determine whether disruption of *VDH1* results in changes in expression of other genes which could also be involved in morphogenesis. We are also examining a *VDH1* homologue we identified in the plant pathogen *V. albo-atrum*, to determine if differences in gene sequence or expression may account for the characteristic lack of MSC in this close relative of *V. dahliae*.

482. Biological function of the genes *ras* and *tpk* in the fungus *Schizophyllum commune*. N. Knabe, E. Kothe. FSU Jena, Jena, Germany

It has been shown that cAMP affects normal development of fruit bodies in *S. commune*. A certain concentration of cAMP causes many fruit bodies to stop morphogenesis at an early stage. Those fruit bodies that continue development have abnormal or no gills. Therefore, the role of cAMP-dependent signal transduction is investigated in the wood-rotting homobasidiomycete *S. commune*. Since the components of cAMP signaling cascades are well conserved in eukaryotes, two components are investigated. One is the G-protein Ras which is known to induce adenylate cyclase in Saccharomyces cerevisiae. An activated Ras is therefore expected to lead to high intracellular cAMP concentration and thus to mimic high external cAMP concentrations. A cDNA sequence of *ras1* from *S. commune* could be used to derive PCR primers to amplify a fragment from genomic DNA. The 1.2kb fragment contains the entire *ras1* gene and shows 98% amino acid homology to Ras1. Both dominant negative and constitutive Ras will be used to analyze Ras function in sexual development and fruit body formation in *S. commune*. The second protein under investigation is cAMP dependent protein kinase A which is translating the signal from differing cAMP levels to regulation of transcription factors by phosphorylation. For *tpk*, encoding the catalytic domain of protein kinase A, a fragment was cloned and sequenced. The fragment of 3.4 kb contains almost the entire *tpk* gene which shows 60% amino acid identity to the yeast protein Tpk2. A knock-out mutant will be constructed to investigate the role of cAMP-dependent signaling in sexual development and fruit body formation in *S. commune*.

483. The *Aspergillus nidulans* F-Box Protein GrrA is required for Ascosporogenesis. Sven Krappmann[1], Branka Medic[1], Nadja Jung[1], Rolf Prade[2], and Gerhard H. Braus[1]. [1] Department of Molecular Microbiology and Genetics, Georg-August-University Goettingen, Germany. [2]Department of Microbiology and Molecular Genetics, Oklahoma State University, USA

Aspergillus (*Emericella*) *nidulans* is a well-established model organism to study elementary processes of differentiation. A Negative Subtraction Screening procedure was performed with the aim to isolate transcripts that are expressed at elevated levels during cleistothecia formation. Among them, one EST that is specifically up-regulated at the end of fruit body development could be identified. The deduced amino acid sequence of the equivalent full-length cDNA shows similarity to the *S. cerevisiae* F-box protein Grr1p and the *A. nidulans* GrrA protein complements the morphology defect of a yeast *grr1D* mutant strain. The *grrA* locus was deleted in a wild-type background and the resulting mutant strain was characterised thoroughly: whereas no specific phenotype is obvious with respect to growth capacities, mitosis, asexual sporulation or cleistothecia development, there are no ascospores to be found within the ripe fruiting bodies of the *grrAD* mutant. As specificity factors of so-called SCF ubiquitin ligase complexes, F-box proteins influence protein stability and turnover. Conclusively, the *grrA* gene product links ascosporogenesis in *A. nidulans* to proteasome-executed protein degradation mediated by the SCF^{GrrA} complex.

484. A potential role of basidiolipids as physiological ligands of galectins during sexual development of the mushroom *Coprinopsis cinerea*. Martin Waelti[1], Piers Walser[2], Rinaldo Bertossa[3], Cristina Villalba[1], Ursel Kuees[4], Markus Aebi[1] and Markus Kuenzler[1]. [1]Institute of Microbiology, ETH Zuerich, Switzerland; [2]Present address: Institute for Molecular Bioscience, University of Queensland, Australia; [3]Present address: Department of Genetics, University of Groningen, The Netherlands; [4]Institute of Forest Botany, Georg-August Universitaet Goettingen, Germany

Galectins, a family of beta-galactoside-binding lectins with a characteristic carbohydrate-recognition-domain (CRD) signature, have been implicated in cell differentiation, cell adhesion, cancer, apoptosis, and pre-mRNA-splicing in animals. Interestingly, the protein family is absent from archaea, bacteria, plants and most fungi but is found in mushrooms. The temporal and spatial expression pattern of two isogalectins, CGL1 and CGL2, in the ink cap mushroom *Coprinopsis cinerea* suggests a role for these galectins in fruiting body formation. We are aiming to identify the physiological ligand(s) of the *C. cinerea* galectins in order to understand their molecular function in this developmental process. Our preliminary results point to a subfamily of glycosyl-inositol-phosphoryl-ceramides (GIPCs) containing beta-galactosides as candidate ligands. These so-called “basidiolipids” have been described in a number of different mushrooms thereby revealing a remarkable species-to-species variability in their sugar headgroup. At present, we are in the process of isolating basidiolipids from *C. cinerea* and characterizing them both structurally and with regard to their galectin-binding activity. We hypothesize that the binding of developmentally regulated galectins to basidiolipids may play a crucial role in mushroom development.

485. Classical genetics in fruiting body development of *Coprinopsis cinerea*. P. Srivilai, W. Chaisaena and U. Kües. Georg-August-University Göttingen, Institute for Forest Botany, Göttingen, Germany

Coprinopsis cinerea is an excellent model to study fruiting body development in the basidiomycetes. The self-compatible homokaryon AmutBmut with mutations in the *A* and *B* mating type genes serves us to isolate mutants in fruiting body formation. Genetic analysis of such mutants turned out to be difficult by lack of compatible co-isogenic monokaryons. Progenies from crosses of both homokaryon AmutBmut or AmutBmut mutants with foreign strains often show an extreme variety in fruiting behaviour (lack of initiation, block of fruiting at various developmental stages, defects in fruiting body maturation, defects in sporulation), indicating that in most monokaryotic strains the natural sets of fruiting genes are suboptimal. Therefore, by repeated back-crossing, we generated wildtype monokaryons with different mating types that are co-isogenic to homokaryon AmutBmut. In crosses with mutants, these strains give inheritance patterns that are possible to interpret. Genetic analysis of some interesting AmutBmut fruiting mutants will be presented. Moreover, we will present observations on influences of mating types on fungal growth.
Our laboratory is supported by Deutsche Bundesstiftung Umwelt (DBU) and scholarships by the Mahasarakham University (to PS) and the Rajamangala Institute of Technology (to WC).

486. Monstrosities under the Inkcap mushrooms. M. Navarro-González, A. Domingo-Martínez, S. Navarro-Gonzáles, P. Beutelmann* and U. Kües. Georg-August-University Göttingen, Inst. Forest Botany, Göttingen and *Johannes Gutenberg-University of Mainz, Inst. General Botany, Mainz, Germany

Inkcaps are a group of basidiomycetes whose mushrooms usually deliquesce shortly after maturation for spore liberation. Until recently, inkcaps were compiled under one single genus *Coprinus*. However, molecular data divided the fungi into four new genera: *Coprinus, Coprinopsis, Coprinellus* and *Parasola* (Redhead et al. 2001). Species within these genera are often difficult to recognize beyond doubt and, most likely, many species are still undescribed. *Rhacophyllus lilacinus* is a mushroom-like anamorph found on horse dung. It carries lysomeres or bulbils (large, round propagules) on lamella and on the upper surface of the cap. Occasional formation of basidiospores in these structures classified the fungus as *Coprinus clastophyllus*, respectively *Coprinopsis clastophylla* (Maniotis 1964). Environmental conditions appear to decide whether the monstrous anamorphs are formed or fruiting bodies that carry four meiospores at the basidia on the lamella. Molecular analysis of ITS sequences confirmed assignment to the genus *Coprinopsis*. The species is closest related to *Coprinopsis stercorea*. Another strain isolated from horse dung forms crippled mushrooms of various shapes (“hunchbacks”, “picked helmets”, “split umbrellas”, “cedar-shape”). So far, this strain could not be identified on the species level. ITS sequencing groups it close to *Coprinellus curtus*. Supported by the DBU and CONACYT.

487. The Role of Protein Myristoylation in Fungal Development. Soochan Lee and Brian D. Shaw. Program for the Biology of Filamentous Fungi, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77803, USA.

In a previous study the *A. nidulans* polarity mutant *swoF1* was found to be mutated in a gene encoded a protein N-myristoyl transferase (NMT). NMTs transfer the 14 carbon fatty acid myristate to the N-terminus of a small group of proteins. This modification allows otherwise cytoplasmic proteins to associate with membranes. We hypothesize that a myrisotylated protein downstream of SwoF plays an important role in growth polarity. Six suppressor of *swoF1* (ssf) mutants have been identified. Genetic analysis has shown that all six mutations are extragenic to *swoF*. At least two distinct, reduced growth phenotypes are observed when each *ssf* mutant is released from the *swoF* background. Interestingly, *ssfA, ssfC* and *ssfD* secrete a red pigment into the medium similar in color to ascoquinone, as made during ascosporogenesis. This is of particular note, since the G alpha proteins GanA, GanB, and FadA are likely targets for myristoylation. The distinct reduced growth phenotype of each mutant allows for cloning by complementation. To date *ssfD* has been complemented using a plasmid based genomic library, but the complementing clone has not been analyzed. Continued analysis of these mutants will be discussed. Progress using an *in vivo* labeling approach to ascertain myrisotylated targets of SwoF will be discussed.

488. Microarray analysis of the *Gibberella zeae* cDNA clones obtained by subtractive hybridization against an isogenic *mat1-2* deletion strain. Seung-Ho Lee[1], Sanghyeob Lee[2], Doil Choi[2], Sung-Hwan Yun[3], and Yin-Won Lee[1]. [1]School of Agricultural Biotechnology, Seoul National University, Seoul 151-921; [2] Korea Research Institute of Bioscience and Biotechnology, Taejeon 305-600; [3]Division of Life Sciences, Soonchunhyang University, Asan, 336-745, Korea

Gibberella zeae is a homothallic ascomycete causing head blight on several cereal crops. Ascospores of this fungus can overwinter within the sexual fruiting body (perithecium) and initiate the primary infection in the next spring. Thus, a greater understanding of sexual development in *G. zeae* is needed for a comprehensive disease control strategy. We have focused on identifying the genes specifically controlled by *MAT* gene, a master regulator of sexual reproduction in *G. zeae*. To do that, we employed suppression subtractive hybridization between self-fertile *G. zeae* Z3643 and an isogenic strain deleted for *MAT1-2* (*delmat1-2*). In total, 1,000 expressed sequence tags (ESTs) were generated from the cDNA subtraction library and 378 EST unigenes were identified. To select the genes expressed under control of *MAT1-2*, we performed a cDNA microarray analysis using the unigenes. Among them, 228 (61.1%) clones were highly expressed in strain Z3643, when grown on mating plates, but not in *delmat1-2*. These included the genes similar to a *Ste12*-like transcription factor, Grg1 protein involved in glucose-repression, a glutamate carboxypeptidase-like protein1, a NADPH-cytochrome P450 reductase, and to several hypothetical proteins. Differential expression of the 15 genes from this collection was confirmed by Northern blot analysis.

489. Identification of a sclerotial-specific storage protein gene (*ssp1*) and transcript profiling of sclerotial development by microarray in *Sclerotinia sclerotiorum*. Moyi Li, Andrew R. Hutchens and Jeffrey A. Rollins. University of Florida, Dept. of Plant Pathology, Gainsville, FL

Sclerotial development plays a key role in the life cycle of *Sclerotinia sclerotiorum*. To gain an understanding of this regulatory process we are isolating and characterizing genes differentially expressed during the transition from vegetative growth to sclerotial development. The first recipient of this analysis is a gene encoding a sclerotial-specific storage protein (*ssp1*), initially identified by peptide sequencing and translated EST comparison. SSP1 makes up 35%-40% of the total protein in mature sclerotia and is also abundant in apothecial tissue. Northern analysis revealed that the *ssp1* transcript accumulated in all stages of sclerotial developmental but was absent in both apothecial tissue and vegetative hyphae. These results suggest that *ssp1* transcript accumulation is exclusive to the sclerotial stages of the life cycle, but that the SSP1 protein is translocated to the apothecium during carpogenic germination. We have hypothesized that SSP1 functions as a carbon and nitrogen source during apothecial development. Degradation of SSP1, however, is not detected in sclerotia or apothecia. An *ssp1* gene deletion mutant will be constructed to further investigate its function during sclerotial development. To identify other genes differentially regulated during sclerotial development, we have constructed a small scale, 500 element, cDNA microarray. Hybridization analysis indicated that ~60 genes were up-regulated and ~50 genes were down-regulated greater than 2-fold during sclerotial initiation relative to vegetative mycelial growth. We are investigating the functions of these sclerotia-related genes by sequence analysis and gene deletion.

490. Sexual reproduction between partners of the same mating-type in *Cryptococcus neoformans*. Xiaorong Lin[1], Christina M. Hull1[5], and Joseph Heitman[1, 2, 3, 4]. Departments of Molecular Genetics and Microbiology[1], Medicine[2], Pharmacology and Cancer Biology[3], the Howard Hughes Medical Institute[4], Duke University Medical Center, Durham, North Carolina 27710 Current address[5]: Depts. of Biomolecular Chemistry and Medical Microbiology & Immunology, University of Wisconsin - Madison Medical School, Madison, WI 53706-1532

C. neoformans is a ubiquitous bipolar basidiomyceteous fungus with a defined sexual cycle involving haploid cells of alpha and **a** mating-type, yet the vast majority of clinical and environmental isolates are alpha cells. Sexual reproduction plays an important role in evolution, yet given the preponderance of alpha strains in the environment it has been a mystery how *C. neoformans* mates to produce meiotic progeny. *C. neoformans* grows as a budding yeast in culture and in the infected host, but it undergoes a filamentous dimorphic transition during the sexual cycle to produce spores, the suspected infectious agent. Alpha strains can also filament and sporulate by a process known as monokaryotic fruiting, which resembles mating but was previously thought to be asexual with no ploidy change. We discovered that key hallmarks of mating occur during monokaryotic fruiting, including a change in ploidy (diploidization) and meiosis (ploidy reduction, frequent recombination). Efficient monokaryotic fruiting requires mating machinery and the key meiotic factor Dmc1. These studies reveal how sexual reproduction can occur in nature between isolates of the same mating-type.

491. A mutation in *Aspergillus nidulans* chaperonin subunit, *cctA* results in a tip splitting, polarity maintenance phenotype. Melissa Long and Brian D. Shaw. Program for the Biology of Filamentous Fungi, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77803, USA.

The *A. nidulans cctA1* temperature sensitive mutant is defective in polarity maintenance when grown at restrictive temperature (39C). When germinated at 39C *cctA1* extends initially a germ tube more or less normally but the conidium continues to expand isotropicaly, resulting in a swollen basal cell twice the size of wild type. The most profound phenotype of the *cctA1* mutant at 39C is dichotomous branching at the growing tip of the germ tube. The mutant is complemented by a plasmid containing An5713.2 as designated in The Broad Institute *A. nidulans* genomic database. Transposon insertion within An5713.2 disrupts the ability of this clone to complement the cctA1 mutant. The An5713.2 predicted protein encodes a chaperonin subunit with 65% identity (e value 0) to *S. cerevisiae* protein Cct7. The chaperonin is a complex made up of two each of eight subunits in a double ring that assists in folding of approximately 10% of newly synthesized proteins. Though the exact make up of the substrates of this complex is not fully understood, the best studied to date are the cytoskeletal proteins, actin and tubulin. Homologs of all eight *S. cerevisiae* chaperonin subunits are found in the *A. nidulans* genome. It is hypothesized that a cytoskeletal defect leads to the *cctA1* tip splitting phenotype. Analysis of this hypothesis will be discussed.

492. Role of Cdc42 and Rac1 for cytokinesis and morphology in *Ustilago maydis*. Michael Mahlert, Verena Welteke and Michael Bölker. University of Marburg, Dept. of Biology, Karl-von-Frisch Str. 8, D-35032 Marburg

We are studying signalling cascades regulating morphology and cytokinesis in *Ustilago maydis*. Small GTPases of the Rho/Rac family act as molecular switches. The GTP bound form stimulates downstream effectors.
We were able to construct a viable *cdc42* deletion mutant which only displayed a cell separation defect. Another small GTPase, Rac1, shows high sequence similarity to Cdc42. Deletion of the *rac1* gene resulted in a phenotype, which is clearly different from that of *cdc42* deletion strains: *rac1* mutants are unable to bud, instead they multiply by fission at a centrally located septum. This indicates, that Rac1 plays a role in the regulation of cell polarity and bud formation. A double mutant, in which *cdc42* is deleted and *rac1* is expressed under the control of an inducible promoter displays a lethal phenotype under repressing conditions. Therefore Cdc42 and Rac1 share at least one essential function.
Interestingly, overexpression of wildtype Rac1 induced the formation of long filaments similar to that formed by dikaryotic cells. Expression of constitutive active Rac1Q61L resulted in a lethal phenotype which is characterized by the formation of large vacuolated cells. Currently we are screening for synthetic lethal mutants which depend on either Rac1 or Cdc42. We aim to identify novel components of the cascades containing Rac1 and Cdc42.

493. Differential Regulation of Fruitbody Development and Meiosis by the Unlinked Aspergillus nidulans Mating Type Loci. Karen Y. Miller, Autumn Nowell and Bruce L. Miller. University of Idaho.

The haploide genome of the homothallic fungus Aspergillus nidulans possesses both mating type genes previously described for other heterothallic and homothallic filamentous fungi. matA encodes the HMG box protein and homolog of N.crassa MAT-a (general MAT-2). matB encodes the alpha box protein and homolog of N.crassa MAT-A-1 (general MAT-1-1). Homologs of MAT-1-2 and MAT-1-3 do not exist in A.nidulans. Unlike other fertile filamentous fungi, the A. nidulans mating type genes are located on separate chromosomes and freely segregate as independent loci. The A.nidulans mating type loci are not idiomorphs and suggests that homothallism in A. nidulans may not have arisen via a recombination event in a heterothallic predecessor. Vegetative growth and conidiation are not affected in single or double matA/matB mutant strains. A matA deletion strain differentiates only reproductive foci (proto-cleistothecia) and Hulle cells. A few mature fruiting bodies are formed after three weeks (~1% of wild type levels). However, the internal hyphae of these cleistothecia undergo greatly delayed and limited differentiation of ascospores. By contrast, developmental timing and numbers of cleistothecia in a matB deletion strain are similar to wild type. However, there is a complete failure of meiosis, the internal tissue eventually disintegrates and the cleistothecia lyse. matA/steA and matB/steA double mutant strains have phenotypes similar to a steA mutant. This suggests that steA functions upstream of the mating type genes, or is essential for amplification of mating type gene expression. The medA gene (medusa) is also required for self-fertility in A.nidulans. medA mutants abort at Hulle cell and foci formation. medA encodes a transcription factor that directly regulates matA expression and directly, or indirectly, regulates matB expression.

494. The JAMM motif of the COP9 signalosome is essential for *Aspergillus nidulans* sexual development. Krystyna Nahlik[1], Silke Busch, Elke U. Schwier, Gerhard H. Braus[2]. Institut of Microbiology and Genetics, Georg-August-Universitaet, 37077 Goettingen, Germany

The COP9 signalosome (CSN) is a conserved multiprotein complex, playing an essential role in development of many eukaryotes, including the filamentous fungus *Aspergillus nidulans*. The deduced amino acid sequences of four recently identified *A. nidulans* genes: *csnA, csnB, csnD* and *csnE*, show high identities to the respective subunits of higher eukaryotes. Considering the conservation of the proteasome-related machinery in *A. nidulans*, it provides an easily amenable model for studying the broad array of CSN developmental functions.
Deletion of either *csnD* or *csnE* results in pleiotropic phenotypes affecting formation of fruit bodies during sexual development, light-dependent signalling and secondary metabolism. We are trying to determine how exactly the observed phenotypes arise: are they associated with disturbed deneddylation activity of COP9 (the removal of NEDD8 tag from cullin, a subunit of ubiquitin ligase), are kinases associated with CSN responsible for these effects, or is it a combination of both?
We have addressed the first part of this question by point mutagenesis of the *csnE*, containing the JAMM metalloprotease motif, which has been shown to convey the deneddylation activity. Mutant strains with *csnE* bearing point mutations in the codons for H134, H136 and D145 of the JAMM motif were constructed. The JAMM mutant phenotype is identical with that of the *DcsnE* strain, indicating that the COP9 deneddylation activity alone is essential for sexual development in *A. nidulans*. Analysis of cullin neddylation state in these mutants is underway.

495. Characterization of transcription factors from the filamentous ascomycete *Sordaria macrospora* and their implications on fruiting-body development. Nicole Nolting, Stefanie Poeggeler. Ruhr-University, 44780 Bochum, Germany

Fungal morphogenesis is regulated by a complex network of various transcription factors. In this study we analysed the mating-type HMG-domain gene *Smta-1* and the putative MADS-box gene *mcm1* of the homothallic ascomycete *Sordaria macrospora*. We constructed deletion- and overexpression strains.
The mating-type gene *Smta-1*, encoding an HMG-domain protein, was shown to be involved in fruiting-body morphogenesis, since deletion of *Smta-1* resulted in mutant strains that are impaired in perithecia development.
The *S. macrospora* MCM1 protein is a member of the MADS-box family of transcription factors. MADS-box proteins are combinatorial transcription factors that often derive their regulatory specificity from other DNA binding factors. So far, little is known about the role of MADS-box proteins in fruiting-body development. A yeast two hybrid-analysis and *in-vitro* analyses revealed that the *S. macrospora* MCM1 protein has the capability to interact with the mating-type protein SMTA-1. Overexpression of *mcm1* led to an increased production of perithecia. Thus, our results suggest an involvement of the *S. macrospora* transcription factors SMTa-1 and MCM1 in fruiting-body development.

496. Identification of a fruiting body-specific protein from pyrenomycetes. Minou Nowrousian[1], Markus Piotrowski[2], Ulrich Kück[1]. [1]Lehrstuhl für Allgemeine und Molekulare Botanik and [2]Lehrstuhl für Pflanzenphysiologie, Ruhr-Universität Bochum, 44780 Bochum, Germany

Native polyacrylamide gel electrophoresis of soluble proteins from several Neurospora species and related ascomycetes identifies an acidic protein that is preferentially present in perithecial extracts (Nasrallah and Srb, Proc Nat Acad Sci USA 70: 1891-1893, 74: 3831-3834). We have now isolated a protein from *Sordaria macrospora* showing similar biochemical features: The protein appears as a strong band on native gels of perithecial extracts but is absent from mycelial protein preparations. Its molecular weight was determined by SDS gel electrophoresis to be ~24 kDa which is similar to the corresponding *N. crassa* protein. We have obtained peptide sequences from the *S. macrospora* protein by mass spectroscopy and were able to identify the corresponding gene. The protein does not have significant homology to any characterized proteins; but interestingly, the corresponding gene was identified previously by microarray hybridization as being strongly downregulated in three developmental mutants from *S. macrospora* that carry mutations in signal transduction proteins or transcription factors and do not form any mature fruiting bodies (Masloff et al. Genetics 152: 191-199, Pöggeler and Kück Eukaryot Cell 3: 232-240). Taken together, these data indicate that the isolated protein might play a role in fruiting body development of pyrenomycetes.

497. Spatial and temporal expression of laccase in *Coprinopsis cinerea* using galectin promoters. J. K. Pemmasani, R. Velagapudi, S. Kilaru, K. Kaur, P. Hoegger, U. Kües. Georg-August-University of Göttingen, Institute for Forest Botany, Göttingen, Germany

Fruiting body development in homobasidiomycete *Coprinopsis cinerea* is a complex, poorly understood cellular process. Galectins are beta-galactoside binding lectins expressed during initiation and development of fruiting bodies in *C. cinerea*. The proteins are encoded by two genes, *cgl1* and *cgl2*. *cgl2* is expressed in the mycelium under dark conditions at the initiation of fruiting body development till the end of primordia formation, whereas expression of cgl1 starts later with the light-induced formation of secondary knots, compact hyphal aggregates (Boulianne et al. 2000, Microbiology 146: 1841; Bertossa et al. 2004, FGB 41: 1120). In this study, we establish the *C. cinerea* laccase gene *lcc1* as a reporter gene to study spatial and temporal regulation of the *cgl1* and *cgl2* promoters. Within agar plates, promoter activity is locally detected by conversion of the laccase substrate ABTS into a colored substance. Within fruiting bodies, promoter activity is detected by enzyme tests with isolated tissues. Enzymatic tests for specific detection of promoter activities within tissues of intact structures are to be developed. The methods will be useful for later analysis of other promoter activities during fruiting body development.
Our laboratory is financially supported by the Deutsche Bundesstiftung Umwelt (DBU).

498. Pheromones and pheromone receptors of the homothallic ascomycete *Sordaria macrospora*. Severine Mayrhofer, Stefanie Poeggeler. Ruhr-University, 44780 Bochum, Germany

The genome of the homothallic filamentous ascomycete *Sordaria macrospora* is predicted to encode two pheromone precursors and two seven-transmembrane pheromone receptors. The deduced proteins of the pheromone precursor genes *ppg1* and *ppg2* are structurally similar to the alpha-factor precursors and a-factor precursors of the yeast *Saccharomyces cerevisiae*, respectively. The products of the pheromone receptor genes *pre2* and *pre1* display significant sequence similarity with the *S. cerevisiae* Ste2p alpha-factor receptor and Ste3p a-factor receptor, respectively. Deletion of pheromone genes *ppg1* and *ppg2* does not affect vegetative growth or fertility of *S. macrospora*. However, deletion of pheromone receptor genes resulted in delayed fruiting body and ascospore development. Double mutants e.g. deltapre2/deltappg2 produce only a drastically reduced number of perithecia and ascospores, thus implying an involvement of pheromones in fruiting body development of homothallic ascomycetes.
In order to analyse the functionality of pheromone /receptor pair of the homothallic *S. macrospora*, we used a heterologous yeast assay. The *S. macrospora* alpha-factor-like pheromone precursor PPG1 was shown to be processed to an active pheromone by yeast MAT alpha cells. The *S. macrospora* PRE2 receptor was demonstrated to be a G-protein coupled receptor that can substitute for the endogenous Ste2p receptor in yeast MATa cells.

499. Ras Gap 1 of the homobasidiomycete *Schizophyllum commune* regulates growth rate, hyphal growth orientation and fruitbody formation. Daniela Schubert and Erika Kothe. Institute of Microbiology, Friedrich-Schiller-University, Jena, Germany.

The white rot fungus *Schizophyllum commune* is used for the analysis of mating and sexual development in homobasidiomycete fungi. We isolated the gene *gap1* encoding a GTPase-activating protein for Ras. Disruption of *gap1* should therefore lead to strains accumulating Ras in its activated, GTP-bound state and to constitutive Ras signaling. Haploid *delta gap1* monokaryons of different mating types did not show alterations in mating behaviour in the four different mating interactions in a tetrapolar mating system. Instead, growth rate in *delta gap1* monokaryons was reduced about 25 % and about 50 % in homozygous *delta gap1/delta gap1* dikaryons. Monokaryons, as well as homozygous dikaryons carrying the disrupted *gap1* alleles exhibited a disorientated growth pattern. This failure of maintenance of growth direction was especially obvious in dikaryons during clamp formation as hook cells failed to fuse with the peg beside them. Instead, the dikaryotic character of the hyphae was rescued by fusion of the hooks with nearby developing branches. *delta gap1/delta gap1* dikaryons formed increased numbers of fruit body primordia, whereas the amount of fruit bodies was not raised. Mature fruit bodies formed no or abnormal gills. No production of spores could be observed.

500. Characterization of subunit one and two of the *A. nidulans* COP9 Signalosome. Elke U. Schwier, Oliver Draht, Silke Busch, Krystyna W. Nahlik, Gerhard H. Braus. Institut für Mikrobiologie und Genetik, Georg-August-Universitaet Goettingen (GER)

The COP9 Signalosome (CSN) is a highly conserved protein complex critical to the accurate development of numerous multicellular organisms. It shares structural similarities with the lid subcomplex of the proteasome and the translation initiation factor 3 (eIF3). Recently two subunits of the COP9 Signalosome were isolated in the filamentous ascomycete *A. nidulans*. Deletion of either *csnD* or *csnE* resulted in pleiotrophic phenotypes affecting the sexual cycle, secondary metabolism and light signaling.
We report the isolation and characterization of *csnA* and *csnB* from *A. nidulans*. The *csnA* coding sequence of 1497 bp is interrupted by three introns resulting in a protein of approximately 55,7 kDa. The approximately 54,2 kDa Protein CSNB is encoded by a DNA sequence of 1670 bp. Both deduced amino acid sequences contain a PCI domain and show high identity to the analogous CSN subunits of higher eukaryotes. Deletion of either *csnA* or *csnB* resulted in viable strains with hyphae appearing partly red. The sexual cycle is initiated but fruit body formation is blocked at the stage of primordia. These defects are identical to those found for the *csnD* and *csnE* deletion strains. We conclude that *csnA* and *csnB* code for the first two subunits as defined by database searches of the COP9-Signalosome in *A. nidulans*. Strains carrying a *csnA::gfp* fusion are constructed to verify where the protein is localized at different developmental stages.

501. Multiple roles of a heterotrimeric G protein gamma subunit in governing growth and development of *Aspergillus nidulans*. Jeong-Ah Seo, Kap-Hoon Han, and Jae-Hyuk Yu
Dept. of Food Microbiology and Toxicology, & Food Research Institute, University of Wisconsin-Madison, 1925 Willow Drive, Madison, 53706 USA

Vegetative growth signaling in the filamentous fungus *Aspergillus nidulans* is primarily mediated by the heterotrimeric G protein composed of FadA (Galpha), SfaD (Gbeta) and a presumed Ggamma. Analysis of the *A. nidulans* genome identified a single gene named *gpgA* encoding a putative Ggamma subunit. The predicted GpgA protein consists of 90 amino acids showing 72% similarity with yeast Ste18p. Deletion (D) of *gpgA* resulted in reduced vegetative growth and lowered asexual sporulation. Moreover, despite highly elevated Hülle cell production, the D*gpgA* mutant was unable to produce sexual fruiting bodies (cleistothecia) in self-fertilization and was severely impaired with cleistothecial development in outcross, indicating that GpgA is required for sexual development. Developmental and morphological defects caused by deletion of *flbA* encoding an RGS protein controlling FadA-signaling were suppressed by D*gpgA*, suggesting that GpgA primarily functions in vegetative growth signaling. However, deletion of *gpgA* could not bypass the need for *fluG* in asexual sporulation, indicating that GpgA indirectly affects conidiation. We propose that GpgA defines the Ggamma subunit constituting the FadA-SfaD::GpgA heterotrimer, and is required for normal vegetative growth and proper asexual/sexual developmental progression. Effects of deletion of *gpgA*, *sfaD* and/or *fadA* on mRNA levels of remaining G protein subunits are also presented.

502. *Aspergillus nidulans* Morphogenesis Mutant, swoK1, is defective in a gene that encodes an RNA binding protein. Srijana Upadhyay and Brian D. Shaw. Program for the Biology of Filamentous Fungi, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77803, USA.

The *Aspergillus nidulans swoK1* mutant is defective in polarity maintenance when grown at restrictive temperature (38C). Upon germination, the *swoK1* mutant extends a primary germ tube that swells to an enlarged, non-uniform cell with pronounced wall thickenings. The mutant is fully restored to wild type growth when transformed with a plasmid containing the gene An5802.2 as designated in The Broad Institute *A. nidulans* genomic database. The locus in the *swoK1* mutant contains a single base deletion (G) 545 bp downstream from the predicted start codon that results in a frame-shift that is predicted to terminate in a stop codon 18 amino acids down stream of the lesion. The SwoK predicted protein contains an N-terminal RRM (RNA Recognition motif) and a highly repetitive C-terminus with SR repeats and RD repeats. The SwoK protein is 43% identical to the *S. pombe* protein Srp1. The proteins each contain the N-terminal RRM and in the SR repeats, but SRP1 does not contain the RD repeats. In *S. pombe* Srp1 is both an RNA splicing factor and responsible for RNA translocation out of the nucleus. Full length homologs of this protein are found in the filamentous fungal genomes of *N. crassa* and *M. grisea*. The possible role of this protein in polarized development will be discussed.

503. How many hydrophobins does a mushroom need? R. Velagapudi, S. Peddireddi*, P.J. Hoegger, A. Majcherczyk, A. Polle and U. Kües. Georg-August-University Göttingen, Institute for Forest Botany and NHN*, Göttingen, Germany

Within the genome of the dung fungus *Coprinopsis cinerea* (http://www.broad.mit.edu) we identified 34 potential type I hydrophobin genes by multiple BLAST searching, using known hydrophobin sequences from the NCBI data base. Compared to other fungi, this is the largest hydrophobin group known so far. For example, there are 20 different genes in the white-rot fungus *Phanerochaete chrysosporium* (http://genome.jgi-psf.org), two in the smut Ustilago maydis and none in the human pathogen *Cryptococcus neoformans* (http://www.broad.mit.edu). In *C. cinerea*, the closely related genes *Coh1* to *Coh7* are clustered together in the genome as the closely related genes *Coh29* to *Coh34*, suggesting them to have been arisen via duplications. Other less related genes are spread within the genome either singly or in groups of two or three. Within vegetative mycelium and fruiting bodies, we detected multiple stage-specific hydrophobins of about 10 kDa and different proteins were identified by LC-MS. Within phylogenetic trees, there is no defined correlation of *C. cinerea* proteins with fruiting- and mycelium-specific hydrophobins from other basidiomycetes. Further protein analysis and gene expression studies will verify how many of the multiple hydrophobin genes are functional at the different stages of development.
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504. Two putative $Zn(II)_2Cys_6$ transcription factors, RosA (repressor of sexual development) and NosA (no sexual development) trigger early developmental decisions in the filamentous fungus *Aspergillus nidulans*. Kay Vienken[1], Reinhard Fischer[2]. [1]Biochemistry; Max-Planck-Institute for terrestrial Microbiology; Marburg; [2]Applied Microbiology, TU Karlsruhe; Germany

Morphologic differentiation processes such as spore production in fungi are largely controlled by stage-specific expression of regulatory proteins. We have analyzed two putative $Zn(II)_2Cys_6$ transcription factors in the filamentous fungus *A. nidulans*. One gene, *rosA*, encodes a 713 amino acid long protein transiently expressed during asexual rather than sexual development.
Expression was also induced after carbon starvation. Deletion of the gene caused the fungus to initiate sexual spore production under non-appropriate conditions, such as carbon-starvation or in liquid culture. Subcellular localization of a RosA-GFP fusion protein suggested that shuttling of the factor between the cytoplasm and the nucleus may be important for the function. In summary, RosA is involved in the transduction of environmental conditions into developmental decisions. The second regulator, NosA, is in contrast to the negative regulator RosA, essential to induce sexual development. The expression of the gene appeared to be constitutive suggesting posttranscriptional regulation of NosA activity. To identify putative target genes for the two important regulators we performed DIGE-2D-gel electrophoresis with subsequent peptide mass fingerprinting for protein identification.

505. Withdrawn

506. COT1, POD6 and LRG1 are required for hyphal elongation in *Neurospora crassa*. N. Vogt, N. Rabanizada, G. H. Braus, and S. Seiler. Inst. of Microbiology & Genetics, Goettingen, Germany

In a screen for mutants defective in hyphal morphogenesis, we have identified temperature-sensitive mutants in *cot-1, pod-6* and *lrg-1* that result in the cessation of hyphal elongation and induction of hyperbranching at restrictive temperature. COT1 is related to Rho kinases. POD6 is a novel protein with similarity to the STE20 superfamily, and the GTPase activating domain of LRG1 suggest an involvement of Rho proteins in hyphal elongation. *cot-1;pod-6* double mutants are identical to the parental strains, while *cot-1;lrg-1* and *pod-6;lrg-1* are synthetic lethal. Mutations in the dynein complex and in the RNA binding protein GUL1 were identified as common suppressors for all three mutants. Also, enhanced sensitivity to oxidative stress and partial suppression of the growth defect through osmotic stabilizers was observed for all mutants. COT1 and POD6 colocalize in a vesicular network throughout the hypha. This distribution is dependent on the activity of both dynein and conventional kinesin. Domain analysis of LRG1 indicates that the LIM and GAP domains are both necessary for wild type growth. Overexpression of Rho1 result in full suppression of the *lrg-1* growth defect. In vitro GAP assays are in progress to characterise the function of the GAP domain at a molecular level. Taken together, these results suggest that COT1/POD6 are acting together in a functional complex, but that this complex needs the activity of an independent LRG1/RHO1 dependent pathway to coordinate hyphal morphogenesis.

507. Growth and developmental mutants of *Aspergillus fumigatus*: Comparative forward genetics. Jae-Hyung Mah, Kap-Hoon Han, Greg Flygt and Jae-Hyuk Yu. Food Microbiology and Toxicology, University of Wisconsin-Madison

Aspergillus fumigatus (AF) is the most prevalent airborne pathogen, which causes severe invasive aspergillosis in immunocompromised patients. While the available genome sequences of AF and the model fungus *Aspergillus nidulans* (AN) permits comparative reverse genetic studies, gene-deletion or overexpression may not be sufficient to unveil the molecular mechanisms underlying species-specific physiological outcomes. In fact, disruption of two major developmental regulators *flbA* and *fluG* in AF provided evidence that, although AF and AN share conserved G protein-mediated growth signaling, AF has distinct sporulation processes. To further dissect upstream regulation of growth and development in AF, we carried out forward genetic studies and isolated four groups of AF mutants exhibiting: 1) enhanced colony growth 2) moderately fluffy-autolytic 3) wet-melting spores and 4) non-conidial fluffy phenotypes. Mutations responsible for the mutant groups 1 and 2 were mapped within AF-GpaA (Galpha) or AF-*flbA*. In conjunction with deletion analyses of AF-*fluG* and AF-*flbA*, identification of these mutations further supports the hypothesis that AF-GpaA and AF-FlbA coordinate balanced growth and sporulation, but AF has distinct and persistent sporulation mechanisms. These findings suggest that genes required for AF sporulation may only be identified via forward genetic approach. Transformation-based gene identification is underway. Specific mutations, associated phenotypic changes and speculated genetic model are presented.

508. *GzRum1*, a putative transcription regulator required for ascospore development in *Gibberella zeae*. Hee-Hyung Kim[1], Theresa Lee[2], Yin-Won Lee[3], and Sung-Hwan Yun[1]. [1]Div. of Life Sciences, Soonchunhyang Univ., Asan, 336-745; [2]Gene expression team, NIAB, RDA, Suwon 441-707; [3]School of Agricultural Biotechnology, Seoul Nat¡¯l Univ., Seoul 151-921, Korea

Gibberlla zeae is a homothallic ascomycete with ubiquitous geographic distribution. This fungus infects several cereal crops and causes disease symptoms such as scab and head blight. The importance of *G. zeae* ascospores in disease development had been recognized since the sexual spores that can overwinter in a sexual fruiting body (perithecium) cause early infection in the next spring. Our goal is to identify and characterize the genetic traits involved in sexual development by *G. zeae*. In this report, we show the function of a cDNA clone (*GzRum1*) obtained by cDNA subtraction analysis of the wild-type *G. zeae* Z3643 against an isogenic *mat1-2* deletion strain. *GzRum1* exhibited a high sequence similarity to a transcriptional repressor encoded by *Rum1* in *Ustilago maydis*, which is essential for sexual spore formation in *U. maydis*. Targeted deletion of *GzRum1* caused no phenotypic changes in the *GzRum1*-deletion mutants except ascospore formation. The mutant produced normal-looking perithecia but no ascospores. RNA blot analysis revealed that expression of *GzRum1* strongly increased only during the perithecial stage and that the *GzRum1* transcript was not detected in the *mat1-2* deletion strain, indicating that transcription of *GzRum1* is regulated by *MAT1-2* in *G. zeae*.

Biochemistry and Secondary Metabolism

509. Characterization of the genomic region, controlling biosynthesis of host-specific AAL-toxins, on the conditionally dispensable chromosome of the tomato pathotype of *Alternaria alternata* . Yasunori Akagi[1], Hajime Akamatsu[1,4], Mikihiro Yamamoto[2], Takashi Tsuge[3], Hiroshi Otani[1] and Motoichiro Kodama[1]. [1]Plant Pathology Lab, Dept Agriculture, Tottori Univ, Tottori 680-8553, Japan. [2]Okayama Univ. [3]Nagoya Univ. [4]Current address: Dept Plant Pathology, WSU, USA.

AAL-toxins are host-specific toxins produced by *A. alternata* tomato pathotype (*A. alternata* f. sp. *lycopersici*), the causal agent of Alternaria stem canker disease of tomato, which cause severe necrosis on susceptible tomato cultivars. AAL-toxins and fumonisins of the maize pathogen *Gibberella moniliformis* are structurally related to sphinganine and termed sphinganine-analogue mycotoxins. A 120 kb genomic region on a BAC clone that contains the AAL-toxin biosynthetic (*ALT*) gene cluster in the tomato pathotype was sequenced and compared with corresponding sequences of the fumonisin biosynthetic (*FUM*) gene cluster in *G. moniliformis*. The genomic region includes 19 putative ORFs and 12 of these show similarity to the genes in the *FUM* gene cluster. These genes include fungal Type I PKSs (*ALT1*), cytochrome P450 monooxygenases, dehydrogenases, aminotransferases, ABC transporters and longevity assurance factors. However, the order of the genes in the *ALT* gene cluster is different from that in the *FUM* gene cluster. In addition, the *ALT* gene cluster resides on a 1.0 Mb conditionally dispensable chromosome (CDC) found only in the pathogenic and AAL-toxin-producing strains of *A. alternata* and homologues of the genes were not detected in nonpathogenic strains of *A. alternata* . Genomic sequences of *ALT1* and another PKS gene located outside of the *ALT* gene cluster, both of which reside on the CDCs in the tomato pathotype strains were compared to each other in tomato pathotype strains collected worldwide. This revealed that the sequences of the genes located on the CDCs, from strains with different geographical origins, are identical. Conversely, the sequences of genes located on chromosomes other than CDCs between the same strains are not identical, indicating that the origin of the CDCs might differ from that of the other chromosomes in the tomato pathotype. We propose a hypothesis in which the ability to produce AAL-toxins could be potentially distributed among *A. alternata* strains by horizontal transfer of the CDCs. This could provide a possible mechanism whereby new pathogens arise in nature.

510. Inhibition of acetyl-CoA carboxylase impairs appressorial melanization and lipid degradation during infection-related morphogenesis in *Colletotrichum lagenarium*. Makoto Asakura[1], Carine A. J. Masson[2], Alison M. Hill[2], Tetsuro Okuno[1], and Yoshitaka Takano[1]. [1]Kyoto University, Kyoto, Japan. [2]University of Exeter, Exeter, UK.

Soraphen A is known to be a specific inhibitor of acetyl-CoA carboxylase (ACC). We investigated effects of soraphen A on infection mechanism of *Colletotrichum lagenarium* that causes cucumber anthracnose. Growth of *C. lagenarium* on nutrient medium was completely inhibited in the presence of 0.5 ug/ml of soraphen A. In contrast, conidia germinated and developed appressoria normally even in the presence of 2.0 ug/ml of soraphen A. However, soraphen A inhibited melanization of appressoria, suggesting that ACC newly synthesizes malonyl-CoA for appressorial melanization. During appressorium formation and maturation, lipid bodies were gradually degraded. Interestingly, non-melanized appressoria treated with soraphen A retained abundant lipid bodies, suggesting inhibition of lipid-body degradation. This phenomenon was not observed in non-melanized appressoria treated with melanin inhibitors, carpropamid and tricyclazole that inhibit conversion of scytalone to vermelon and vermelon to 1.8-DHN, respectively. However, disruption of *PKS1* specifically involved in polyketide synthesis for melanin inhibited breakdown of lipid bodies. Thus, inhibition of polyketide synthesis for melanin as well as inhibition of ACC attenuate breakdown of lipid bodies. These suggest that the melanin biosynthesis pathway affects regulation of lipid degradation during infection-related morphogenesis in *C. lagenarium*.

511. Metabolic Gene Cluster Silencing in *Aspergillus nidulans*. Jin Woo Bok*, Daan Noordermeer, and Nancy P. Keller. Department of Plant Pathology, University of Wisconsin - Madison, 1630 Linden Drive, Madison, Wisconsin 53706, USA

In contrast to primary metabolism, the genes involved in secondary metabolism are clustered in fungi. Recently a nuclear protein, LaeA, was found to be required for the transcription of several secondary metabolite gene clusters in *Aspergillus nidulans* (Bok and Keller 2004). Here we show that regulation is confined to secondary metabolite clusters and not other defined metabolic clusters including proline, nitrate and ethanol utilization clusters. One of these clusters contains the positive regulatory (i.e. *aflR*) and biosynthetic genes required for biosynthesis of sterigmatocystin (ST), a carcinogenic toxin. Analysis of ST cluster expression indicates LaeA regulation of the cluster is location specific as transcription of genes bordering the ST cluster are unaffected in a delta *laeA* mutant and placement of a primary metabolic gene, *argB*, in the ST cluster resulted in *argB* silencing in the delta *laeA* background. ST cluster gene expression was remediated when an addition copy of *aflR* was placed outside of the cluster but not when placed in the cluster. Site specific mutation of a S-adenosyl methionine (AdoMet)-binding site in LaeA generated a delta *laeA* phenotype suggesting the protein to be a methyltransferase. We present a model of LaeA involvement in chromatin regulation of secondary metabolite gene clusters.

512. Characterization of *Fusarium verticillioides* alternatively spliced and other ESTs. Daren W. Brown, Robert H. Proctor, Robert R.A. Butchko, Foo Cheung*, Christopher Town*, and David F. Kendra. Mycotoxin Research Unit, NCAUR, USDA/ARS, 1815 N. University St., Peoria, IL 61604. *The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850

Fusarium verticillioides is a pathogen of maize worldwide and produces fumonisins, a family of mycotoxins that have been associated with several animal diseases and cancer in humans. The fumonisin biosynthetic genes are located in a co-regulated 15-member gene cluster spanning 43 kb of genomic sequence. In order to identify genes that regulate fumonisin biosynthesis and that are involved in the *F. verticillioides*-maize interactions, we generated over 87,000 expressed sequence tags (ESTs) that represent 11,119 unique sequences from nine cDNA libraries prepared from the fungus grown under fumonisin-inducing and non-inducing conditions and in the presence of maize tissues. Of particular interest, we found that 78 of the 700 ESTs that match portions of the 15 fumonisin genes were alternative splice forms (ASFs). Most ASFs are predicted to yield truncated proteins due to stop codons and/or frameshifts in the retained introns or the altered sequence due splice events utilizing an alternate 3' border. We found that the ASFs appeared to be differentially expressed as more were present in libraries derived from older cultures. This, coupled with the high frequency of occurrence of some ASFs suggests they serve a biological role. We have begun to examine the occurrence of ASFs in culture over time using microarrays. The physiological importance of alternative splicing in fungi has not been determined. Understanding their role in fumonisin biosynthesis may open up new avenues to develop strategies to limit fumonisin contamination of agricultural commodities.

513. Isolation, cloning and characterisation of three glutathione-s-transferases in *Aspergillus fumigatus*. Claire Burns, Rachel Geraghty, Kevin Kavanagh and Sean Doyle National Institute for Cellular Biotechnology, National University of Ireland, Maynooth, Ireland.

Aspergillus fumigatus is a severe fungal pathogen of immunocompromised patients commonly treated with antifungal agents such as amphotericin B. However, treatment is often ineffective and infection frequently leads to death. We postulate that detoxification enzymes such as glutathione-s-transferases (GST) may play a role in survival of the fungus during infection, and would thus provide a novel therapeutic target.
Several putative GSTs were identified from the *A. fumigatus* genome. Three of these genes, namely *gst1*, *gst2* and *gst3*, have been cloned and heterologously expressed, yielding proteins of 29-30 kDa. The recombinant proteins exhibited GST activities against CDNB of 0.025 U/mg, 0.006 U/mg and 0.004 U/mg, and glutathione peroxidase activities against cumene hydroperoxide of 0.145 U/mg, 0.025 U/mg and 0.019 U/mg respectively.
Gst2 and *gst3* were basally expressed, and were induced 4-fold and 10-fold in the presence of CDNB, and 3-fold and 5-fold in the presence of hydrogen peroxide. *Gst1* was not basally expressed, and was induced by CDNB only. Experiments are ongoing to examine expression in the presence of other xenobiotics. Preliminary results when induced with amphotericin B indicate possible downregulation of *gst2* and *gst3* and upregulation of *gst1*.

514. *In silico* subtraction of *Fusarium verticillioides* EST libraries to identify potential transcriptional regulators of the *FUM* gene cluster. Robert A.E. Butchko, Daren W. Brown and Robert H. Proctor. Mycotoxin Research Unit, NCAUR, USDA/ARS, 1815 N. University St., Peoria, IL 61604

Fumonisins are polyketide-derived mycotoxins produced by the maize pathogen *Fusarium verticillioides*. These toxins can disrupt sphingolipid metabolism in animals, cause diseases in horses and swine, and have been associated with cancer in laboratory rodents. A cluster of fumonisin biosynthetic genes (*FUM*) has been described in *F. verticillioides*. Conspicuously absent from this *FUM* gene cluster is a transcription regulatory gene. Previous Northern analysis indicated that *FUM* genes are not expressed at early times points (e.g. 24 hr) in GYAM medium but are highly expressed at later time points (e.g. 96 hr). We have prepared EST libraries from *F. verticillioides* GYAM cultures, and the presence/absence of *FUM* gene ESTs in 24- and 96-hr libraries is consistent with the differential expression observed in the Northern analyses. Further comparison of the libraries revealed the presence of a number of EST's with similarities to transcription factors and activators, DNA binding proteins, and zinc finger proteins in the 96-hr library but not in the 24-hr library. We are investigating whether the genes corresponding to these ESTs regulate *FUM* gene expression. To do this, we have disrupted the putative regulatory genes and developed a rapid screen to assay *FUM* gene expression. Understanding the transcriptional regulation of *FUM* genes should provide information that can be used to control fumonisin contamination in maize.

515. Isolation and sequence analysis of polyketide synthase genes from three Fusaria commonly found in corn. Bryce Callighan[1], Keith Johnson[1], Daren W. Brown[2], [1] Bradley University, Biology Department, Peoria IL. [2] USDA-ARS-NCAUR, Mycotoxin Research Unit, Peoria IL.

Mycotoxins are fungal secondary metabolites that often contaminate grains following plant infestation. Synthesis of many mycotoxins (e.g. fumonisins and fusarins produced by *Fusarium verticillioides*) is initiated by a member of a family of enzymes called polyketide synthases (PKSs). Degenerate primers were developed that target a highly conserved portion of genomic sequence that encodes the PKS ketosynthase (KS) domain. The first objective of the research was to isolate KS gene fragments from *F. verticillioides*, *F. proliferatum*, and *F. subglutinans* genomic DNA. Based on the genomic sequence of *F. verticillioides* and *F. graminearum*, between 10 and 20 PKS genes are expected per genome. To date five novel KS fragments from both *F. proliferatum* and *F. subglutinans* have been isolated. We conducted a phylogenetic analysis of the new KS sequences and KS sequences from fungal PKSs with known as well as unknown function. We identified a number of species specific PKS genes as well as PKS genes that appear to be shared by multiple Fusarium. In addition, the unique sequence data has provided us tools to help distinguish between species in mixed cultures.

516. Cloning, characterization and expression of a polyketide synthase gene involved in monacolin k biosynthesis from *Monascus* sp. Yi-Pei Chen[12], Li-Ling Liaw[2], Ming-Der Wu[1], Chun-lin Wang[1], Ching-Ping Tseng[2], Gwo-Fang Yuan[1]. [1]Bioresource Collection and Research Center, Food Industry Research and Development Institute. [2]Department of Biological Science and Technology, National Chiao Tung University.

Monacolin k, cholesterol serum synthesis inhibitor, is a secondary metabolite synthesized by polyketides from *Monascus*. In this study, a BAC (Bacterial Artificial Chromosome) clone, mps01, was screened from the mpb01 BAC library constructed with *Monascus* sp. BCRC 38072 genomic DNA. The putative monacolin k biosynthesis gene cluster was found in mps01 clone, genomic sequencing and Northern blot analysis showed that nine putative genes for monacolin k biosynthesis were located within a 41-kb region and were transcribed when monacolin k was produced. The deduced amino acid sequences encoded by the nine genes, designated *mkA*¡V*mkI*, sharing similarities of over 54% with lovastatin gene cluster contained in *Aspergillus terreus* were assumed to be involved in monacolin k biosynthesis. The *mkA* gene encoding nonaketide synthase and *sfp* gene, a phosphopantetheinyl transferase required to convert the expressed apo-PKS to its holo form and obtained from *Bacillus subtilis*, were coexpressed in *Escherichia coli*. Novel polyketide compounds produced in the transformant were determined by LC-ESIMS and found at wavelength of 360 nm. Further study on the structure of these polyketides will be presented.

517. Genetic analyses of a peptide synthetase gene from the insect pathogen *Metarhizium anisopliae*. Yong-Sun Moon (1), Stuart B. Krasnoff (2), John D. Vandenberg (2), Donna M. Gibson (2), and Alice C.L. Churchill (1,3), Boyce Thompson Institute (1), USDA-ARS, Plant Protection Research Unit (2), and Department of Plant Pathology, Cornell University (3), Ithaca, NY, USA

Metarhizium species are at the forefront of efforts to develop entomopathogenic fungi as insect biocontrol agents. Yet we have an incomplete understanding of the biological and genetic factors that make them effective. We have focused our efforts on understanding the roles of toxins as virulence factors in fungal-insect interactions. The principal toxins produced in fermentation by *M. anisopliae* are the destruxins, a large family of cyclic depsipeptides, which are predicted to be synthesized nonribosomally by a large multifunctional enzyme called a peptide synthetase (PS). We targeted for further study a PS gene fragment (ma267) identified by Freimoser et al. (2003) as an EST that was expressed after 24 hr of fungus growth on insect cuticle-containing medium. We determined that ma267 detects DNA polymorphisms that correlate with relative levels of *in vitro* destruxins production in several *M. anisopliae* isolates. Additionally, ma267 gene expression is positively correlated with *in vitro* destruxins production. We disrupted the ma267 PS gene by *Agrobacterium tumefaciens*-mediated transformation and homologous recombination and identified several genetically stable knockout (KO) transformants. All KO and ectopic transformants examined exhibited normal growth rates, colony phenotypes, and wild type levels of *in vitro* destruxins production, suggesting that the ma267 PS gene is not involved in destruxins production in *M. anisopliae*. Bioassays against beet armyworm are in progress to determine if disruption of the ma267 PS gene affects pathogenicity of the fungus. An understanding of the role of toxins in pathogenicity is essential for enhancing *M. anisopliae* as a biocontrol agent and to confirm its safety against non-target organisms.

518. Nitrogen source and pH influence aflatoxin production by a previously unrecognized *A. flavus* morphotype . P. J. Cotty, K.E. Kobbeman, J.E. Mellon, T. Feibelman, K. F. Cardwell, and K.E. Ehrlich. USDA, ARS, Division of Plant Pathology and Microbiology, University of Arizona, Tucson, AZ

An *Aspergillus* that produces B aflatoxins and elongate tan sclerotia was isolated from soils collected in North America, Asia, and West Africa. Phylogenetic analysis of the *aflJ/aflR A. flavus*. All isolates belonged to the same vegetative compatibility group and shared greater than 99% sequence similarity. This morphotype (morphotype P) produced elongate sclerotia, up to 5 mm in length, with a bulbous base and reduced melanin. In contrast to other aflatoxin-producing *A. flavus*, P morphotype isolates produced 8 to 190 fold more B aflatoxins in a medium with nitrate as the sole nitrogen source than in media with either ammonium or urea as sole nitrogen sources. Other *A. flavus* isolates and isolates of *A. nomius* and *A. parasiticus* produced either similar quantities or more aflatoxins in ammonium-based medium than in nitrate based medium. Low aflatoxin production in ammonium-based medium resulted from sensitivity to low pH created during fermentation on ammonium. Buffering with either succinate or citrate resulted in increased aflatoxin production. Aflatoxin production by both the P morphotype and *A. parasiticus* (NRRL 2999) was greater in nitrate than in ammonium at pH 2.5. However, at pH 7.5, both fungi initially produced greater quantities of aflatoxins on ammonium medium. Variation in pH sensitivity may cause variation among *A. flavus* isolates in aflatoxin production. The P morphotype may be useful for studying interrelationships among sclerotial morphogenesis, aflatoxin biosynthesis, and melaninization.

519. Cloning, Expression, and Knockout of Polyketide and Tetramic Acid Synthase Genes from Fungi. Russell J Cox, Thomas J. Simpson FRS, Colin Lazarus, Andy Bailey, Kirstin Eley, Song Zhongshu, Deirdre Hurley, Frank Glod, Thomas P. Nicholson and Ying Zhang. University of Bristol, School of Chemistry, Bristol UK

A rapid cloning procedure has been developed for obtaining polyketide synthase genes from fungi which are associated with the production of particular compounds. The cloning and analysis of genes involved in fungal squalestatin and fusarin biosynthesis will be discussed.

520. Expression analysis of the cross-pathway control genes *cpc1* and *cpc2* in *Acremonium chrysogenum*. Jacqueline Dreyer and Ulrich Kück. Algemeine & Molekulare Botanik, Ruhr- University Bochum, D- 44780 Bochum, Germany

The biosynthetic pathway and external factors influencing antibiotic production in *Acremonium chrysogenum* have already been elucidated. However, the genetic alterations leading to differences in cephalosporin C production between wild type and overproducing strains are still poorly understood. There are several distinct reports indicating that gene regulation at higher level is responsible for the alteration in cephalosporin C production. It has been shown that the gene regulation mediated through glucose in the overproducing strains is clearly different from the one in the wild type strain (Jekosch K, Kück U, 2000 Curr Genet 37: 388-395). Furthermore, several transcription factors have been isolated which play a role in the regulation of gene expression in cephalosporin C biosynthesis (Schmitt EK et al. 2004 In: Molecular Biotechnology of Fungal beta-Lactam Antibiotics and Related Peptide Synthases. Series: Advances in Biochemical Engineering / Biotechnology 88: 1-43).
We have confirmed the presence of two genes *cpc1* and *cpc2* in *Acremonium chrysogenum* which are key transcription factors in the amino acid biosynthetic pathway. Preliminary studies on their gene expression by northern analysis and real-time PCR demonstrate that these genes are regulated. The data from wild type and semi- producer strain are compared. A knock-out strain is being generated to investigate the role of *cpc1* in secondary metabolism.

521. The aflatoxin biosynthesis cluster gene, hypA is involved in conversion of versicolorin A to sterigmatocystin. Kenneth C. Ehrlich, Beverly Montalbano, Steve Boue, and Deepak Bhatnagar, Southern Regional Research Center/ARS/USDA, PO Box 19687, New Orleans LA 70124

The conversion of polyketide metabolite, versicolorin A, to sterigmatomcystin (ST) probably requires five different enzymatic steps: deoxygenation, Baeyer-Villiger oxidation, lactone cleavage, oxidative decarboxylation, and methylation. The proteins encoded by verA and ver-1 are probably involved in the first two steps. We now report that the aflatoxin biosynthesis cluster gene, hypA, also encodes an enzyme involved in the conversion. Knockout plasmid vector constructs were prepared in pUC18 and used to transform A. parasiticus BN009E niaD-. Five transformants were isolated which accumulated versicolorin A and no longer produced AF. Northern blot and PCR analysis showed that only hypA was disrupted in these transformants and that other genes (verA and ver-1) were expressed at normal levels. Feeding studies confirmed that ST and O-methylST, but not versicolorin A, were efficiently converted to aflatoxins, in the knockout transformants. The enzymatic role of HypA in the conversion has not been established. HypA and its gene have probable membrane-binding and dehydrogenase domains based on a motif search of the GenBank database. We hypothesize that HypA is involved in the necessary lactone cleavage and rearrangement steps which convert the oxidized anthraquinone to the xanthone.

522. Isolation of an Epichloë Endophyte Gene Cluster Involved in Ergovaline Biosynthesis. Damien Fleetwood[1,2], Aiko Tanaka[2], Barry Scott[2] and Richard Johnson[1]. [1]AgResearch Limited, Palmerston North, New Zealand; [2]Institute of Molecular Biosciences, Massey University, Palmerston North, New Zealand

Epichloë endophytic fungi are obligate symbionts that colonise the intercellular spaces of temperate pasture grasses of the subfamily Pooideae. This symbiosis is vitally important to agriculture as secondary metabolite alkaloids produced by the endophyte protect the grass host from many herbivores. However some endophyte alkaloids are toxic to grazing animals. One such toxic alkaloid is ergovaline, an ergopeptine that causes toxicosis in cattle and sheep grazing tall fescue and perennial ryegrass pastures in the USA and New Zealand. Here we present the isolation, by degenerate PCR, of a non-ribosomal peptide synthetase gene from *Neotyphodium lolii* (a common perennial ryegrass endophyte closely related to *Epichloë* species) that has homology to the Lps2-encoding gene involved in ergopeptine biosynthesis from *Claviceps purpurea*. Screening of an *N. lolii* genomic library provided several overlapping clones which have been sequenced. In addition to the putative NRPS gene orthologue (here named *lps*B), a second gene has been identified that is also found in the *C. purpurea* ergopeptine gene cluster, a putative oxidoreductase *oxr*A. This, along with expression and taxonomic distribution data, provides very strong evidence that we have isolated the ergovaline biosynthetic gene cluster from *N. lolii* and progress towards the isolation of the full gene cluster is underway. To determine the function of *lps*B a targeted gene replacement has been constructed and the mutant introduced into perennial ryegrass to determine the *in planta* chemical phenotype. Similar experiments are underway with *oxr*A.

523. Functional analysis of the polyketide synthase genes in *Gibberella zeae*. Iffa Gaffoor[1], Daren W. Brown[2], Ron Plattner[2], Robert Proctor[2], Weihong Qi[1], and Frances Trail[1]. [1]Michigan State University and [2]USDA, Peoria IL.

Type I Polyketide Synthases (PKSs) are multidomain enzymes responsible for synthesizing a gamut of compounds with varied functions. From the genomic sequence of the filamentous fungus Gibberella zeae (anamorph *Fusarium graminearum*) we identified fifteen putative polyketide synthase genes. We have disrupted each of these genes, along with an additional gene that shares some similarity with PKSs but which we think does not encode a PKS. Individual disruption mutants have been analyzed for traits such as vegetative growth, mycotoxin and pigment production, perithecium production, ascospore discharge and pathogenicity. From these analyses, we have identified PKS genes responsible for zearalenone, fusarin and aurofusarin biosynthesis and biosynthesis of the black perithecial pigment. Expression analysis of these genes under varied culture conditions revealed that they are differentially expressed. While two PKS genes appear to be expressed under most of the conditions tested others are not expressed under any of these conditions. Further analysis of the mutants and identification of the polyketide compounds synthesized by the PKSs will enable us to assign functions to the remaining PKS genes and elucidate their role in the life cycle of *G. zeae*.

524. Molecular Genetics and Evolutionary Aspects of the Alkaloid Pathway in *Claviceps purpurea*. T. Haarmann, and P. Tudzynski. Institute of Botany, WWU Muenster, Schlossgarten 3, D-48149 Muenster, Germany

We isolated and cloned genes organized in a cluster of 68.5 kb that could mediate pathway specific steps of the ergot alkaloid biosynthesis. Expression studies showed that all cluster genes are coregulated and that they are only activated under alkaloid producing conditions. The cluster comprises the gene *cpd1* which encodes the key enzyme DMATS, four NRPS (*cpps1-4*), several oxygenases and oxidoreductases and other ORFs not characterized so far. Targeted inactivation of one NRPS (*cpps2*) led to an ergopeptine-nonproducing mutant which – unlike the parent producer strain – accumulated D-lysergic acid. *Cpps2* was shown to encode the monomodular lysergyl-peptidyl-synthetase 2 (LPS2) responsible for the activation of D-lysergic acid. Knock-out experiments with the gene *cpP450-1* led also to an ergopeptine-nonproducing mutant which instead accumulated agroclavine, indicating the involvement of the gene product in the formation of the precursors of D-lysergic acid (e.g. elymoclavine). Another aim is to compare different strains of *Claviceps* particularly with respect to their potential to produce different types of alkaloids (chemical races). Comparison of the cluster sequences of strain P1 (ergotamine producer) with that of strain ECC93 (ergocristine producer) showed high conservation of most of the cluster genes, but significant variation in the NRPS modules, strongly suggesting that evolution of chemical races of *C. purpurea* is confined to evolution of NRPS module specificity.

525. Crystal structure comparison of two large monofunctional catalases. Wilhelm Hansberg, Adelaida Díaz, Victor-Julián Valdés, Enrique Rudiño-Piñera, Eduardo Horjales. Instituto de Fisiología Celular e Instituto de Biotecnología, Universidad Nacional Autónoma de México, México D. F., México.

Neurospora crassa has two large monofunctional tetrameric catalases, CAT-1 and CAT-3. CAT-1 is associated with non-growing cells and is accumulated in conidia. CAT-3 is inducible and is associated with growing cells. Both catalases are modified by singlet oxygen *in vitro* and *in vivo* and have similar kinetics and resistance to denaturation. The structure of both enzymes was determined by molecular replacement. Heme group in CAT-1 was a mixture of protoheme IX (heme *b*) and an oxidized heme (heme *d*), originated by di-hydroxylation at ring III and subsequent formation of a spirolactone with the propionyl group in C6. The heme *d* probably is formed by singlet oxygen. In addition, CAT-1 has an unusual covalent bond between the sulfur of a cysteine and the beta carbon of the tyrosine that coordinates the Fe(III) of the heme (1). Different to CAT-1, CAT-3 heme is only heme *b* and there is no cavalent bond between the glutamine, equivalent to the CAT-1 cysteine, and the essential tyrosine. Other differences between these enzymes are found at the C–terminal domain. Results indicate that both catalases present different mechanisms to contend with molar concentration of hydrogen peroxide.

1) Díaz A, Horjales E, Rudiño-Piñera E, Arreola R, Hansberg W (2004) Unusual Cys-Tyr covalent bond in a large catalase. J Mol Biol 342:971-985.

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526. Molecular analysis of glycolipid production in *Ustilago maydis*. Sandra Hewald, Beate Teichmann and Michael Bölker. University of Marburg, Dept. of Biology, Karl-von Frisch-Strasse 8, D-35032 Marburg,

Under conditions of nitrogen starvation, *Ustilago maydis* secretes large amounts of amphipathic glycolipids. These surface active compounds can be grouped into two classes: the ustilagic acids and the ustilipids. Ustilagic acids consist of a cellobiose moiety glycosidically linked to the w-hydroxyl group of 15,16-dihydroxy-hexadecanoic-acid. The ustilipids consist of a 4-O-b-D-mannopyranosyl-D-erythritol which is esterified with palmitoic acid and shorter acyl groups.
We used a reverse genetics approach to identify components involved in glycolipid biosynthesis. Potential glycosyltransferases and cytochrome P450 monooxygenases were identified in the genomic sequence of *Ustilago maydis*. Deletion of a glycosyltransferase with similarity to macrolide glycosyltransferases of prokaryotic origin resulted in loss of ustilipid production. Mutant, deleted for a cytochrome P450 monooxygenase of the CYP94A family, lost its ability for ustilagic acid production. Both genes are highly expressed under glycolipid producing conditions.
Therefore we used microarray analysis to identify more genes, which are involved in the glycolipid biosynthesis pathway. Potential candidate genes could be identified and are currently tested.
With the glycolipid defective mutants at hand we are now able to determine the biological functions of these amphipathic substances. We could already show that the secreted glycolipids cause a drastic reduction in surface tension of culture medium.

527. The two interacting transcription factors CPCR1 and AcFKH1 controls cephalosporin C biosynthesis and morphogenesis in *A. chrysogenum* . Birgit Hoff, Danielle Janus, Esther Schmitt and Ulrich Kück. Department of General and Molecular Botany, Ruhr-University Bochum, Universitätsstraße 150, 44801 Bochum, Germany

Acremonium chrysogenum is the main industrial producer of the beta-lactam antibiotic cephalosporin C. A complex regulatory network of transcription factors seems to control the expression of at least seven cephalosporin C biosynthesis genes. In this study, we used the RFX transcription factor CPCR1, which is involved in the transcriptional regulation of the cephalosporin C biosynthesis genes, in a yeast two hybrid screen to identify potential protein interaction partners [1]. A cDNA was identified encoding the C-terminal part of a novel forkhead protein, so called AcFKH1, which is the first characterized member of the forkhead gene family in filamentous fungi. AcFKH1 is characterized by two highly conserved domains, the N-terminal forhead associated domain and the C-terminal DNA-binding domain of the winged helix/forkhead type. The observed interaction between CPCR1 and the C-terminus of AcFKH1 in the yeast system was verified *in vitro* in a GST pulldown assay. Using gel retardation analysis, FKH1 was shown to recognize two forkhead consensus binding sites within the promotor region of the cephalosporin C biosynthesis genes *pcbAB/pcbC*. Additionally, AcFKH1 is able to bind with high affinity to the SWI5-binding site of the yeast FKH2 protein [2].
In a second approach, we investigated the role of the transcriptions factors CPCR1 and AcFKH1 on fungal morphogenesis. We used *cpcR1* and *Acfkh1* knockout strains, multicopy strains and retransformants for detailed light and confocal laser microscopic analysis. From the sum of our investigations we concluded that arthrospore formation and cell separation are dependent on the transcription factors controlling cephalosporin C biosynthesis in *A. chrysogenum*.
[1] Schmitt EK, Bunse A, Janus D, Hoff B, Friedlin E, Kürnsteiner H, Kück U (2004) Eukaryot Cell 3: 121-134
[2] Schmitt EK, Hoff B, Kück U (2004) Gene 342: 269-281.

528. Bimolecular fluorescence complementation (BiFC) – a new tool to visualize protein-protein interactions in living fungal cells. Birgit Hoff and Ulrich Kück. Department of General and Molecular Botany, Ruhr-University Bochum, Universitätsstraße 150, 44801 Bochum, Germany

Here we describe a bimolecular fluorescence complementation (BiFC) assay for direct visualization of protein protein interactions in fungal cells. This *in vivo* approach is based on the formation of a fluorescent complex by two non-fluorescent fragments of the enhanced yellow fluorescent protein (EYFP) brought together by association of interacting proteins fused to these fragments [1]. We have constructed two fungal expression vectors encoding the N- or C-terminus of EFYP with appropriated linker sequences. The application of the expression vectors, and thus of the BiFC technology, was demonstrated by using the two winged helix transcription factors CPCR1 and AcFKH1 from the beta-lactam antibiotic producer *Acremonium chrysogenum* [2]. Both transcription factors bind to promotor sequences of the *pcbAB-pcbC* biosynthesis genes. Only when the full length transcription factors were fused to EYFP fragments, yellow fluorescence was observed due to the bimolecular complementation of both chimeric proteins. No fluorescence was observed in strains producing either CPCR1-EYFPC or AcFKH1-EYFPN in combination with the complementing half-EYFP fragment. Under these conditions, the EYFP fragments do not appear in spatial proximity to each other and, consequently, it is impossible to reconstitute a functional yellow fluorescent protein. The nuclear localization of the protein-protein interaction was verified by staining fungal cells with the nucleic acid dye TOTO-3.
Consequently, the BiFC approach should facilitate the visualization of the subcellular sites of protein interactions in the living fungal cell under conditions that closely reflect the normal physiological environment.
[1] Hu CD, Chinenov Y, Kerppola TK (2002) Mol Cell 9:789-798
[2] Hoff B, Kück U (2004) Curr Genet, in press

529. Visualization of Biochemical Pathways at the Saccharomyces Genome Database. Eurie L. Hong[1], Rama Balakrishnan[1], Karen R. Christie[1], Maria C. Costanzo[1], Kara Dolinski[2], Stacia R. Engel[1], Dianna G. Fisk[1], Jodi E. Hirschman[1], Robert Nash[1], Rose Oughtred[2], Marek S. Skrzypek[1], Chandra L. Theesfeld[1], Gail Binkley[1], Christopher D. Lane[1], Qing Dong[1], Anand Sethuraman[1], Shuai Weng[1], David Botstein[2], J. Michael Cherry[1]. (1) Dept. of Genetics, Stanford University, 300 Pasteur Drive, Stanford, CA, 94305-5120, USA; (2) Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA.

Saccharomyces cerevisiae provides an excellent system for the study of biosynthetic and catabolic pathways and many enzymes involved in these pathways have been identified and characterized. Although there are differences between pathways in *S. cerevisiae* and other organisms (both prokaryotic and eukaryotic), most summaries of pathway information fail to articulate those differences. Using the Pathway Tools software that is developed and maintained by Peter Karp and his colleagues at SRI International, the *Saccharomyces* Genome Database (SGD) has released the Yeast Biochemical Pathways tool (http://pathway.yeastgenome.org/biocyc/). This manually curated resource can be searched to view biochemical reactions and pathways as they occur in *S. cerevisiae*. In addition, pathways can be accessed through hyperlinks on the locus page of enzymes involved in the pathway. This resource also maps data from genome-wide expression analyses onto the pathway overview so that changes in gene expression can be viewed in the context of cellular metabolism. The Yeast Biochemical Pathways tool can be a powerful resource in the classroom, in the analysis of gene expression data, and as a starting point for further investigation of biochemical pathways in *S. cerevisiae* or other fungi. SGD can be accessed at http://www.yeastgenome.org/. SGD is funded by the US National Human Genome Research Institute.

530. Comparative Genomics of a Toxin Biosynthetic Gene Cluster in Filamentous Fungi. Donald M. Gardiner *, Anton J. Cozijnsen, David C. Straney #, Barbara J. Howlett. School of Botany, The University of Melbourne, Victoria, 3010. * Current address: Institute for Molecular Bioscience, The University of Queensland, Queensland 4072. # Department of Cell Biology and Molecular Genetics, University of Maryland, College Park MD 20742 USA

Genes responsible for the biosynthesis of secondary metabolites are typically clustered in filamentous fungi. The origin and evolutionary pressures that maintain such clusters are largely unknown. We have cloned a gene cluster encoding enzymes in the biosynthesis of a toxin, sirodesmin, from *Leptosphaeria maculans*, which causes blackleg disease of canola. Sirodesmin belongs to the epipolythiodioxopiperazine (ETP) class of toxins, which is only produced by fungi and confers toxicity via reduction of a disulfide bond. We are using comparative genomics to determine biosynthetic pathways for ETPs. Putative ETP gene clusters are present in three fungi for which complete genome sequences are available. These are the opportunistic human pathogen *Aspergillus fumigatus*, the rice blast fungus, *Magnaporthe grisea* and the wheat head scab fungus, *Fusarium graminaerum*. *A. fumigatus* makes the ETP gliotoxin, which causes apoptotic and necrotic cell death, as do the distantly related fungi, *Penicillium bilaii* and *Trichoderma virens*. We are attempting to characterise the gliotoxin biosynthetic gene clusters in these fungi. Analysis of the arrangement and sequences of genes in these three clusters may uncover clues about how the clusters evolved.

531. Modelling of Fungal Non-Ribosomal Peptide Synthetases: Prediction of Novel Secondary Metabolites from Fungal Endophytes. Richard Johnson[1], Vic Arcus[2], T. Verne Lee[2], Christine Voisey[1] and Greg Bryan[1]. [1] AgResearch Grasslands Research Centre, Palmerston North, New Zealand. [2] AgResearch Structural Biology Laboratory, University of Auckland, New Zealand.

Neotyphodium lolii and *N. coenophialum* are fungal endophytes that live symptomlessly within the intercellular spaces of perennial ryegrass and tall fescue, respectively. These endophytes confer a number of biotic and abiotic advantages to their hosts, many of which are mediated through the production of fungal secondary metabolites. Biosynthetic pathways involved in secondary metabolism are commonly associated with gene clusters in filamentous fungi and the pathways for several key endophyte secondary metabolites (also see abstracts by Young et al. and Fleetwood et al.), have recently been wholly or partially elucidated.
We are particularly interested in biosynthetic pathways containing non-ribosomal peptide synthetases (NRPSs) since these multi-modular enzymes catalyse the formation of small peptides that are highly diverse in structure and activity. We have identified at least 10 novel NRPS genes from N. lolii, and have isolated corresponding BAC clones that may contain associated gene clusters. Predicting the classes of compounds synthesised from these NRPS associated clusters is presently difficult, especially for fungi where the current homology based models, for adenylation domain binding pocket specificity, do not work well for substrate prediction.
Our research aims to refine these homology based models by determining the high-resolution atomic structure of endophyte peptide synthetase adenylation domains with known substrates (for example lpsA, involved in ergovaline biosynthesis). By combining these models with ligand docking software and knowledge-based scoring we hope to be able to predict the likely substrates of fungal NRPSs.

532. Functional characterization of differentially regulated *Fusarium verticillioides* genes. Nich Jones[1], Keith Johnson[1], Scott E. Baker[2], and Daren W. Brown[3]. [1]Bradley University, Biol. Depart., Peoria IL. [2]PNNL Chem. Biol. Proc. Dev. Group, Richland, WA. [3]USDA-ARS- NCAUR, Mycotoxin Research Unit, Peoria IL.

Fumonisins are secondary metabolites produced by several fungi, including *Fusarium verticillioides*, and are linked to numerous animal diseases, neural tube defects and cancer in humans. *F. verticillioides*, normally an endophyte, can cause several diseases of corn. Damaged and fumonisin contaminated corn result in the loss of millions of dollars each year to farmers world-wide. We reasoned that a better understanding of the genetic regulation of fumonisin production as well as the *F. verticillioides*-plant disease process will enable the development of new strategies to limit fungal damage. Recently, we created nine *F. verticillioides* cDNA libraries which yielded 87,000 ESTs and compiled into 11,119 unique sequences. The presence of numerous unique ESTs in each library suggested that many of them are differentially expressed. We screened the unique sequences and identified a set that may encode for Zn(II)2 Cys6 or Cys2-His2 Zn(II) transcriptional factors. The pattern of differential expression of many of these genes suggest that some may play a role in regulating fumonisin biosynthesis and/or a role in the fungal-plant disease process. We have begun to examine the role these genes may play by creating disruption vectors. We describes the identification of the target genes based on predicted protein sequence, sequence similarities, differential expression, as well as the development of disruption vectors and our efforts to determine the consequences to *F. verticillioides* of the loss of these genes.

533. Characterization of the *mpkB* gene in the model fungus *Aspergillus nidulans*. Navgeet Kaur and Ana M. Calvo. Department of Biological Sciences, Northern Illinois University, Dekalb, Illinois 60115, U.S.A.

In fungi, environmental signals can be transduced into intracellular responses by the action of MAP kinase cascades, which are similar to the ones found in the mammalian cells. Mitogen-activated protein kinases (MAPK) are a family of serine/threonine protein kinases widely conserved among eukaryotes and are involved in many essential developmental processes such as mating, sporulation and pathogenicity. In this research work we are studying the role of *mpkB*, an MAP kinase gene found in the model filamentous fungus *Aspergillus nidulans*. The *mpkB* gene is a homologue of the *FUS3/KSS1* MAP-kinase genes in the yeast *Saccharomyces cerevisiae*. FUS3 regulates mating in response to pheromones in haploid yeast cells and KSS1 regulates filamentous growth in response to nitrogen limitation in diploid yeast cells. In order to elucidate the role of *mpkB* in development and secondary metabolism in *A. nidulans*, we have generated a *mpkB* disruption strain. Loss of MPKB function results in a blockage in biosynthesis of the mycotoxin sterigmatocystin.

534. Characterization of fungal phosphopantetheinyl transferases involved in primary and secondary metabolism. D. Keszenman-Pereyra[1], N. George[2], K. Johnsson[2] and G. Turner[1]. [1]Department of Molecular Biology and Biotechnology, Krebs Institute for Biomolecular Research, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, UK; [2]Institute of Chemical Sciences and Engineering, Ecole Polytechnique Federale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland.

Primary and secondary metabolic pathways in Aspergillus sp utilize 30-50 phosphopantetheinylated proteins with specialized carrier protein (CP) domains. The conversion of apo-CPs to holo-CPs is catalysed by 4'-phosphopantetheine transferases (PPTases). Blast searches of available filamentous fungal genome sequences revealed that each organism has putative genes encoding Fas2 integrated-, AcpS- (postulated to target mitochondrial Acp1) and NpgA- type PPTases. *A. nidulans* NpgA seems capable of activating CPs of non-ribosomal peptide synthetases, polyketide synthases and alpha aminoadipate reductase (homologue of yeast Lys2). To analyse the wide substrate specificity of NpgA, the ORF was amplified by PCR from *A. nidulans* genomic DNA and cloned in frame with N-terminal hexahistidine tag; the PPTase was overexpressed in *Escherichia coli* and purified by nickel affinity chromatography. The wide substrate specificity of NpgA has been confirmed in vitro by using a range of CP substrates and assays. Moreover, co-expression experiments in *E. coli* show that NpgA is relatively efficient for the activation of heterologous substrates. NpgA is the first reported example of a eukaryotic PPTase involved in both primary and secondary metabolism. Phylogenetic relationships of fungal PPTases and their substrates will be discussed.

535. *Aspergillus nidulans* mutants unable to localise glyoxylate cycle enzymes to the peroxisomes are able to utilise acetate. Gillian S. Khew, Sandra L. Murray, Meryl A. Davis and Michael J. Hynes. Department of Genetics, University of Melbourne, Victoria 3010, Australia.

Growth on acetate and fatty acids results in the production of acetyl-CoA which is channeled into the TCA and glyoxylate cycles. The TCA cycle occurs in the mitochondria and the glyoxylate cycle in the peroxisomes. Peroxisomal proteins are directed to the peroxisomes via one of two classes of peroxisomal targeting signals – PTS1 and PTS2. PTS1 targeting occurs via the Pex5 receptor and PTS2 via the Pex7 receptor. These receptors are recycled to the cytosol by Pex6, an ATPase. The *A. nidulans* genes, *acuD* (isocitrate lyase) and *acuE* (malate synthase), encode enzymes specific to the glyoxylate cycle. AcuE possesses a peroxisomal targeting signal 1 (PTS1) whereas AcuD lacks any identifiable PTS1 or PTS2. We are interested in the effects of peroxisomal protein localisation on fatty acid utilisation in *A. nidulans*. A knockout mutant of the *A. nidulans PEX5* orthologue (*pexE*) mislocalises AcuE to the cytoplasm but is able to target AcuD to the peroxisomes. A mutant in the *PEX7* orthologue (*pexG*), however, mislocalises AcuD but is unaffected in peroxisomal targeting of AcuE. A mutant defective in the *PEX6* orthologue (*pexF*) mislocalises both AcuD and AcuE to the cytoplasm but is nonetheless able to utilise acetate. Most of the peroxisomal mutants studied in our laboratory are sensitive to the presence of fatty acids. The growth of the *pexE* mutant is particularly inhibited by the presence of all fatty acids tested, including acetate. A *pexE/pexG* double mutant shows relief of acetate inhibition and is able to grow on acetate.

536. A novel polyketide synthase gene cluster for the biosynthesis of aurofusarin in *Gibberella zeae*. Jung-Eun Kim[1], Kap-Hoon Han[1], Jianming Jin[1], Hun Kim[2], Jin-Cheol Kim[2], Sung-Hwan Yun[3], and Yin-Won Lee[1]. [1]School of Agricultural Biotechnology, Seoul National University, Seoul 151-921; [2]Korea Research Institute of Chemical Technology, Daejon 305-606; and [3]Division of Life Sciences, Soonchunhyang University, Asan 336-745, Korea

Mycelia of *Gibberella zeae*, an important pathogen of cereal crops, are yellow to tan with white to carmine red margins. A screen of insertional mutants of *G. zeae*, generated using a restriction enzyme-mediated integration procedure, resulted in the isolation of mutant S4B3076, which is a pigment mutant. In a sexual cross of the mutant to a strain with normal pigmentation, the pigment mutation was linked to the inserted vector. The vector insertion site in S4B3076 was a *Hin*dIII site 38 bp upstream from an open reading frame (ORF) on contig 1.116 in the *F. graminearum* genome database. The ORF, designated *Gip1* (*Gibberella zeae* pigment mutation 1), encodes a putative laccase. A 30-kb region surrounding the insertion site and *Gip1* contains ten additional ORFs, including a putative ORF identified as *PKS12* that shares about 40% amino acid identity to type I fungal PKS genes. Targeted gene deletion and complementation analyses confirmed that both *Gip1* and *PKS12* are required for aurofusarin production in *G. zeae*. Northern blot analyses revealed that the 10 genes located on the 30-kb region are co-regulated by a putative transcription regulator. This information is the first on the biosynthesis of the red pigment by *G. zeae*.

537. Endopolygalacturonases from *Botrytis cinerea*: biochemical properties and interaction with inhibiting proteins. Geja Krooshof[1], Rob Joosten[1], Harry Kester[1], Ilona Kars[2], Jan van Kan[2], and Jac Benen[1]. [1]Microbiology/Fungal genomics, [2] Phytopathology, Wageningen University, Dreijenlaan 2, 6703 HA Wageningen, The Netherlands.

The phytopathogen *Botrytis cinerea* harbours at least six endopolygalacturonase-encoding (*Bcpg*) genes of which *Bcpg*1 and *Bcpg*2 are required for full virulence. The endopolygalacturonase (BcPG) enzymes degrade pectin, enabling the fungus to breach the plant cell wall. We expressed five BcPG isozymes in *Pichia pastoris*, purified them and studied biochemical properties, such as pH optimum, mode of action, and substrate specificity in detail. BcPG3 shows a rather unusual, broad pH optimum and is the only isozyme fully active at pH 3.5. Polygalacturonic acid is a poor substrate for BcPG1 and BcPG4 as compared to BcPG2, BcPG3, and BcPG6. In contrast to BcPG1, 2, and 4, BcPG3 and BcPG6 show extreme processive behaviour on oligogalacturonides longer than four GalpA residues. Only BcPG3 and BcPG6 are able to hydrolyse GalpA dimers.
Since PG activity is important for fungal virulence, the BcPGs are interesting targets for disease control. Therefore, a range of plant extracts was screened to identify potent polygalacturonase-inhibiting proteins (PGIPs). PGIPs from different plant sources have been purified and their interaction with the BcPG isozymes have been investigated using different techniques. Results on the mode of inhibition and binding will be presented.

538. See abstract number 405

539. Recruitment of primary metabolism genes for the insecticidal loline alkaloid gene cluster in grass-endophytes. Brandi L. Kutil, Charles J. Greenwald, & Heather H. Wilkinson. Program for Biology of Filamentous Fungi, Department of Plant Pathology & Microbiology, Texas A&M University, USA

PLP-binding enzymes are ubiquitous ancient biocatalysts. There are five distantly related lineages (defined by both crystal structure and function). Each of these subfamilies within the superfamily has homologs in the archea, eubacteria and eukaryotic superkingdoms. These enzymes function in many primary metabolism pathways. It is expected that diversification into the five subfamilies of PLP-dependent enzymes occurred prior to divergence of the progenitor of these major kingdoms. Subsequently, radiation of the diversity within each of the gene families has involved evolution of different substrate binding specificities and/or tissue specific expression. Discovery of the loline alkaloid secondary metabolite gene cluster in *Epichloe* and *Neotyphodium* species has revealed some PLP-dependent enzymes specifically involved in lolines production. *LolC* is a homocysteine synthase (*hcs*)-like gene. *LolD* is an ornithine decarboxylase(*odc*)-like gene. We investigated the relationship between each of these *lol* genes, the endophyte primary metabolism genes and publicly available sequences from a variety of eukaryotes, archea and eubacteria. In both gene families the *lol* genes are most closely related to the primary metabolism paralogs from ascomycetes. Thus, we hypothesize the *lol* genes were recruited from within the genome of an ancestor to the endophytes. Funded by USDA-NRI.

540. Molecular Cloning and Characterization of the Polyketide Synthetase Gene from *Monasucs ruber* KCTC6122. Yun Jung Yang, Jae-Yoon Kim[1], In Hyung Lee. Food & Life Science Major, School of Techno Science, Kookmin University, Seoul 136-702, Korea. [1]Biotech Institute, Kookmin University, Seoul 136-702, Korea.

For genetic dissection of biosynthesis of poyketides in *Monascus* sp., the polyketide synthetase (PKS) gene was cloned and characterized. *Monascus* sp. produces various polyketides such as pigments, monakolin K, blood pressure-lowering substance, and nephrotoxic citrinin etc. The degenerate PCR primers were designed based on the conserved amino acid sequences of the KS domain of 14 PKS from various fungi. The PCR products showed over 80% sequence identity to other fungal PKS and they were used for screening of the cosmid library of *Monascus ruber* KCTC6122 and for shotgun cloning. Characterization of the cloned PKS gene is underway and results will be presented.

541. Effects of depleted uranium on the gene expression and germination frequencies of the Arbuscular Mycorrhizal fungus *Glomus intraradices*. Carlos M. Loya, Marijn de Jong, Andrew Bradford, Manjula Govindarajulu and Peter J. Lammers. Dept. Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, USA

Arbuscular mycorrhizal (AM) fungi form obligate symbioses with most plant species. The plants benefit from the association via improved mineral uptake and water relations while the fungus receives fixed carbon from the plant. Depleted uranium contamination of the soil presents a significant environmental problem that until now has not been resolved in an ecologically safe manner. It has been shown that AM fungi, such as *Glomus intraradices*, are able to synthesize metal ligands like citric and oxalic acid that promote depleted uranium (dU) uptake into fungal and plant material. We are attempting to define the genetic and biochemical basis of these processes in order to design a system for dU management. Initial experiments involve monitoring the germination frequency and elongation rates of dU treated germinating spores by microscopic analysis to determine a metal concentration that inhibits germination by 50% (EC50). We next quantified the expression of two fungal genes, Glutathione-S-Transferase (GST) and a putative vacuolar Zn transport protein (Zrc-1), involved in toxic metal responses in three tissues: germinating spores, intraradical and extraradical mycelium. Our results demonstrate significant up-regulation of GST in germinating spores and extraradical mycelium, with minimal changes in Zrc-1. Experiments in progress will follow the expression of these genes after dU addition directly to symbiotic root tissues.

542. Touch Mediated Ca^{2+} signalling in *Neurospora crassa* in response to mechanical perturbation. Marris, P.I., Hickey, P.C. and Read, N.D. Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JH

In the natural envioronment, filamentous fungi respond to contact stimuli (physical surfaces, obstacles and microtopographical features) which elicit various growth and developmental responses. We are investigating the role of Ca^{2+} signalling in response to mechanical perturbation using *Neurospora crassa* expressing codon-optimized aequorin to measure cytosolic free Ca^{2+} ($[Ca^{2+}]_c$). We have found that mechanical perturbation (microinjection of growth medium) of fungal cultures generates reproducible transient increases in $[Ca^{2+}]_c$ in vegetative hyphae (in 18 h-old cultures), germ tubes (3-6 h-old cultures) but not ungerminated conidia. We are presently addressing several questions: (1) Is the Ca^{2+} response to mechanical perturbation dose dependent? (2) Is the $[Ca^{2+}]_c$ continuously elevated in *N. crassa* grown in shake culture or does the Ca^{2+} signalling machinery adapt to continuous mechanical perturbation? (3) Which Ca^{2+}-channels, -pumps and/or antiporters are involved in the response to mechanical perturbation? (4) What physiological/growth/developmental responses to mechanical perturbation can be readily measured in *Neurospora*?

543. Comprehensive functional analysis of all non-ribosomal peptide synthetases in the corn pathogen Cochliobolus heterostrophus. Shinichi Oide and B. Gillian Turgeon. Dept of Plant Pathology, Cornell University, Ithaca NY, 14853

Filamentous fungi have remarkable ability to produce diverse secondary metabolites that are of interest because of their medical, industrial, and agricultural value. Current knowledge of the significance to the producing fungi themselves, however, is limited. We used the corn pathogen, Cochliobolus heterostrophus (Ch) to investigate function of every non-ribosomal peptide synthetase (NRPS). In this study, a set of strains, each carrying a complete deletion of one of the previously identified 11 NPSs (Lee et al, Eukaryotic Cell, in press), was constructed. Double and triple deletion strains were also constructed, by crossing. All strains were examined for their virulence, stress-response, mating, and morphological phenotypes. NPS2, predicted to encode a NRPS involved in siderophore biosynthesis, has a role in sexual development. Deletion of NPS10, which along with NPS6, is conserved in most ascomycetes, causes morphological defects, such as irregular-shaped colonies and reduced asexual sporulation. Deletion of NPS6 leads, concomitantly, to dramatic reduction in virulence on maize and increased sensitivity to oxidative stress. Deletion of NPS6 orthologs from the rice pathogen Cochliobolus miyabeanus, the wheat pathogen, Fusarium graminearum, and Alternaria brassicicola, a pathogen of the model dicot, Arabidopsis thaliana caused the same phenotype as the nps6-deletion strain of Ch. We further examined the functional conservation of NPS6 by introducing the NcNPS6 ortholog from the saprophyte, Neurospora crassa, into the nps6-deletion strain of Ch. NcNPS6 restored virulence of the Chnps6-deletion strain to maize and resistance against oxidative stress, simultaneously. Together, these data demonstrate that NPS6 encodes a NRPS whose product, a presumed small peptide, acts as a defense factor against oxidative stress and has a role in fungal virulence. In summary, these findings suggest that peptides produced by NRPSs play more diverse roles in fungal metabolism than previously thought.

544. The role of peroxisomes in virulence of *Candida albicans*. K. Piekarska and M. van den Berg, G. Hardy, E. Mol and B. Distel. Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

Conclusive evidence has been provided that the ability to switch between yeast and hyphal forms is essential for pathogenesis of *C. albicans*. Once initiated, the hyphal program not only results in the switch to the hyphal morphology to allow penetration of host tissues and in secretion of proteases and phospholipases to break them down, but it also facilitates adaptation to the new environmental conditions, such as the hosts macrophages internal environment. Expression analysis has shown (ref.1) that upon internalization of *C. albicans* by macrophages the yeast upregulates a set of peroxisomal enzymes, notably two glyoxylate cycle enzymes; isocitrate lyase and malate synthase, and enzymes of the beta-oxidation pathway.
Using a PCR-based disruption technique we have constructed *pex5delta/pex5delta* and *pex13delta/pex13delta* deletion strains. Both of these genes are essential for peroxisome formation. To directly test whether peroxisomal beta-oxidation is essential for virulence of *C. albicans*, both copies of the *FOX2* gene encoding the third enzyme of beta-oxidation pathway, were deleted. As expected, none of the constructed double deletion strains was able to grow on oleic acid as a sole carbon source, while the complemented strains showed wild type growth rates on this substrate. Measurements of beta-oxidation activity in these strains revealed that the *fox2delta/fox2delta* strain is the most severely affected in fatty acid beta-oxidation. All strains are currently tested for virulence in a mice model for candidiasis.
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545. Functional analysis of paxilline biosynthesis genes. Sanjay Saikia[1], Emily Parker[2] and Barry Scott[1]. [1]Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand. [2]Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

Paxilline is an abundant indole-diterpene secondary metabolite produced by the filamentous fungus *Penicillium paxilli*. The genes involved in paxilline biosynthesis are organized in a cluster containing at least 5 genes including a geranylgeranyl pyrophosphate synthase (*paxG*), an FAD-dependent monooxygenase (*paxM*), a prenyl transferase (*paxC*) and two cytochrome P450 monooxygenases (*paxP* and *paxQ*). The aim of this research is to validate the role of *P. paxilli* genes in paxilline biosynthesis by biochemical and genetic studies. Radiolabeled precursor feeding studies of *P. paxilli* mutants identified no stable indole-diterpene in *paxG*, *paxM* and *paxC* deletion mutants but identified paspaline and 13-desoxypaxilline in *paxP* and *paxQ* deletion mutants, respectively. Further, a strain lacking the core paxilline cluster genes containing a restriction fragment comprising *paxG*, *paxM*, *paxC* and two other putative genes, *sec23* and *sec25*, produced paspaline demonstrating that this set of genes is necessary and sufficient for the formation of the first stable indole-diterpene in *P. paxilli*. Constructs of wild-type *paxP* and *paxQ* complemented corresponding deletion mutants. The deletion mutants of *paxP* and *paxQ* accumulate paspaline and 13-desoxypaxilline, respectively. Feeding of paspaline to a mutant lacking the core paxilline cluster genes but containing wild-type *paxP* resulted in the synthesis of 13-desoxypaxilline, confirming that PaxP catalyzes the conversion of paspaline to 13-desoxypaxilline, reactions that require demethylation and hydroxylation steps. Similar studies with wild-type *paxQ* showed that PaxQ converts 13-desoxypaxilline to paxilline in a single step hydroxylation.

546. Characterization of the sulfur metabolism network in the phytopathogenic fungi, *Magnaporthe grisea* . Saint-Macary ME, Beaurepaire A, Gagey MJ, Barbisan C, Beffa R, Lebrun MH, Droux M. Laboratoire Mixte CNRS/BayerCropscience -14-20, rue Pierre Baizet – 69009 LYON

Nutritional needs of pathogenic fungi during interaction with their hosts remain poorly described, although some investigations have been undertaken on nitrogen and carbon assimilation (1). Recent studies suggest that pathogenic fungi need sulfur micronutrient during the infection step (2). The fungal sulfur complex pathway includes both genes corresponding to the described metabolism from plant and from yeast (3,4). Sulfate is assimilated and reduced through the common pathway for all autotrophic organisms. Then, reduced sulfur is incorporated for synthesis of two amino acids, cysteine and methionine. In these final steps, filamentous fungi catalyzed both the interconvertion of cysteine to homocysteine (the sulfur precursor for methionine synthesis) through the direct and the reverse transsulfuration pathways. Methionine synthesis from cysteine involves two enzymes of the direct transsulfuration pathway, cystathionine gamma-synthase and cystathionine beta-lyase followed by the methylation step catalyzed by methionine synthase. Cysteine synthesis proceeds through sulfhydration of activated serine but also from homocysteine through the reverse transsulfuration sequence cystathionine beta-synthase and cystathionine gamma-lyase (5).
Studies on sulfur metabolism in the phytopathogenic fungi *Magnaporthe grisea* were developed to understand its role during fungal development on planta. The aim of our work is to identify and to highlight the role of the genes involved in the direct and reverse transsulfuration pathway from the available entire genome of *M. grisea* using genetic and biochemical tools.
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3 Droux M. (2004), *Photosynthesis research*, 79 : 3331-348.
4 Thomas D. and Surdin-Kerjan Y. (1997), *Microbiol. Mol. Biol. Rev.* , 61 : 503-532
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547. Pathway specific regulation of gibberellin biosynthesis in Fusarium fujikuroi – a new type of bZIP transcription factors. Birgit Schönig, Martina Mihlan, Bettina Tudzynski. Institut für Botanik, Westfälische Wilhelms-Universität Münster.

The ascomycete Fusarium fujikuroi produces the economically important phytohormone Gibberellic Acid. One of the genes responsible for GA production is the P450-4 gene encoding the ent-kaurene oxidase. By performing a systematical promotor deletion approach with the P450-4 promotor, a 30 bp region was identified. Its deletion caused a significant decrease in P450-4 transcription. This promotor region was used in a yeast one-hybrid screening to identify proteins interacting with the 30 bp region. A putative transcription factor, GAR (Gibberellic Acid Regulator), has been identified which shows specific binding affinity to the 30 bp region. Its zinc finger domain is most homologous to zinc fingers of "Krüppel like" transcription factors in animals and plants whereas a similar transcription factor had not been described in fungi before. The mutants obtained from a gene replacement experiment show normal mycelium and colony morphology but reduced growth kinetics. Northern blot analysis under GA non-producing and producing conditions revealed a reduced P450-4 transcript level in the mutants. Macroarray experiments will show if GAR affects the transcription of additional genes or if its influence is GA specific. Interestingly, no homologue to GAR could be identified in the genome of the closely related Fusarium graminearum, which also has no GA cluster, whereas in Magnaporthe grisea a GAR homologue and at least two putative GA biosynthetic genes were found.

548. A platform of three gene clusters is required for the biosynthesis of the bioprotective lolitrem alkaloids. Carolyn Young[1], Sanjay Saikia[1], Richard Johnson[2], German Spangenberg[3], Gregory Bryan[2] and Barry Scott[1]. [1]Centre for Functional Genomics, Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand. [2]AgResearch Grasslands Research Centre, Palmerston North, New Zealand. [3]Plant Biotechnology Centre, Primary Industries Research Victoria, La Trobe University, Australia

Lolitrems are potent tremorgenic mycotoxins produced by *Epichloë* and *Neotyphodium* endophytes in association with their host grass *Lolium perenne*. These indole-diterpene alkaloids are responsible for the animal syndrome known as ryegrass staggers and have insecticidal properties. Using candidate genes isolated as ESTs from cDNA libraries combined with chromosome walking from a known lolitrem biosynthesis locus (*ltm*) we have isolated and characterized a ~100 kb platform of three gene clusters required for lolitrem biosynthesis. The clusters are separated by highly repetitive AT-rich sequences that are devoid of open reading frames but contain remnants of retrotransposon sequences. The *ltm* clusters 1 and 2 contain eight genes, seven of which are orthologues of the characterised *P. paxilli* paxilline biosynthesis gene cluster (*pax*). Cluster 3 contains at least two genes, *ltmJ*, a P450 monooxygenase, and *ltmE* a gene fusion of a prenyl transferase and a dimethylallyl tryptophan synthase. All 10 *ltm* genes have similar expression profiles and are highly expressed *in planta* where the production of lolitrem B is most prevalent. When *ltmC* and *ltmM* were placed under the control of a *paxM* promoter they complemented *P. paxilli paxC* and *paxM* deletion mutants, confirming that these two genes are functional orthologues of *paxC* and *paxM*. Disruption of the remaining genes will help elucidate the biochemical pathway for lolitrem biosynthesis.

549. A second D-galactose catabolic pathway in *Hypocrea jecorina*: Involvement of pentose pathway enzymes and implications on cellulase induction. Seiboth B, Gamauf C, Hartl L and Kubicek CP. Institute of Chemical Engineering, TU Vienna, Austria

Hypocrea jecorina is specialized in the degradation of a wide spectrum of plant cell wall polysaccharides. Hemicelluloses are beside cellulose the second most abundant compound in plant cell wall consisting to a major part of sugars such as L-arabinose, D-galactose, and D-xylose. In *H. jecorina* D-galactose is first phosphorylated by galactokinase, while D-xylose and L-arabinose are metabolized by an interconnected pathway consisting mainly of NADPH-linked reductions and NAD-linked oxidations. Strains defective in galactokinase, convert D-galactose via a second path similar to the pentose catabolic pathways and use some of their enzymes. We have biochemical and genetic evidence that D-galactose is converted to galactitol by a NADPH dependent reduction catalyzed mainly by D-xylose reductase, an enzyme of the D-xylose metabolism. L-arabinitol 4-dehydrogenase, an enzyme of L-arabinose metabolism, catalyzes then the oxidation of galactitol. A third pentose pathway enzyme involved is xylitol dehydrogenase which catalyzes a subsequent step, the oxidation of D-sorbitol to fructose. We also will show that both D-galactose catabolic pathways identified are essential for full cellulase induction on lactose and will discuss the importance of both pathways for inducer formation.

550. Identification and characterization of novel type III polyketide synthases in *Aspergillus oryzae*. Yasuyo Seshime[1], Praveen Rao Juvvadi[1], Isao Fujii[2], Katsuhiko Kitamoto[1]. [1]Department of Biotechnology, University of Tokyo, Tokyo, Japan. [2]Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan

Chalcone synthases (CHSs) are prominent members of type III polyketide synthase (PKS) superfamily, and are known to play a vital role in the biosynthesis of plant phenylpropanoids and flavonoids. By virtue of their agricultural and pharmaceutical values, products of type III PKSs have received significant attention in recent years. Although the distribution of type III PKSs has always been considered to be restricted to either plants or bacteria, the present study reveals the existence of type III PKS-encoding genes in an industrially useful fungus, *Aspergillus oryzae*. While *A. oryzae* genome data mining revealed 4 putative CHS-like sequences (*csyA*, *csyB*, *csyC* and *csyD*), reverse transcription polymerase chain reaction indicated the expression of 3 genes (*csyA*, *csyB* and *csyD*). In addition, we confirmed the presence of type III PKS-encoding genes in other filamentous fungi by using the available fungal genome databases. Phylogenic analysis revealed the distinction of fungal type III PKSs from those of bacteria and plants. Interestingly, the other species of Aspergilli (*Aspergillus nidulans*, *Aspergillus fumigatus*) lacked these genes. Significantly, *A. oryzae* alone contained higher number of putative chalcone synthase homologues in comparison to *Neurospora crassa* (1), *Fusarium graminearum* (1), *Magnaporthe grisea* (2) and *Phanerochaete chrysosporium* (3). Disruption of these novel PKS genes in *A. oryzae* is being pursued to elucidate their function in *A.oryzae*.

551. Asparaginase Genes from *Aspergillus nidulans*. Ann L. Onton and Patricia M. Shaffer, Department of Chemistry, University of San Diego, San Diego, CA, 92110, USA

L-asparaginase is an amidohydrolase that catalyzes the hydrolysis of asparagine to aspartic acid and ammonia. Asparaginases are classified into two categories, type I and type II, the latter being regulated. Aspergillus nidulans has two asparaginase genes, *apnA* (on chromosome II) and *ahrA* (on chromosome VIII). The enzyme expressed by the *ahrA* gene is categorized as a type II asparaginase [Shaffer et al. (1988) *Mol. Gen. Genet.* **212**, 337-341].
With the help of Cereon Genomics, LLC (subsidiary of Monsanto) and Whitehead Institute (WI) databases we obtained the sequences of both asparaginase genes. From these sequences we prepared PCR primers and used them with genomic DNA [wild type A4(Glasgow)] to produce nucleotide sequences (1214 nucleotides containing the *ahrA* gene and 706 nucleotides containing the *apnA* gene), as well as for a mutant of each.
Next we prepared labeled sequences for each gene and probed a UniZap cDNA library. We have the cDNA for the *ahrA* gene and are in the process of obtaining the cDNA for the *apnA* gene. The expression of each of these genes is in the planning stages. Since type II asparaginases are used as a cure for childhood acute lymphoblastic leukemia (ALL), this research may have some pharmaceutical significance. We are grateful to Graduate Women in Science for funding and Mark Caddick scientific input.

552. Withdrawn

553. Characterization of metal binding sites in cell wall of *Neurospora crassa*. T. Naga Sowjanya and P. Maruthi Mohan. Department of Biochemistry, Osmania University, Hyderabad-500 007 (A.P.), INDIA

Our previous studies showed that significant levels of calcium and magnesium are present on cell wall fraction of N.crassa. Further, the role cell wall calcium in storage function and maintaining the structural integrity was demonstrated. Based on the above work an improved cell wall preparation method to remove SDS and membrane contamination was used to study binding of metal ions. Ca (3 moles 100 mg-1) and Mg (1.5 moles 100 mg-1) binding was distinct between pH 5 - 6 without competitive effects. The bound metals could be desorbed with dilute HCl or EDTA and rebinding could be demonstrated. Co and Cu binding (3 and 4 moles 100 mg-1) displaced 30% of Ca and 50% of Mg respectively. Presence of NaCl (0.5 M) inhibited 30% of Ca binding but Mg binding was not affected. Glutaraldehyde cross-linked walls bound less Ca (30%), while Mg binding was unaffected. Modification of carboxyl groups of cell walls with carbodiimide resulted in 25% loss of Ca binding, while Mg binding was totally abolished. Enzymatic treatment of cell wall (glucanase, trypsin, chitinase and alkaline phosphatase) caused significant loss of proteins and metal binding capacity. Alterations in Co binding to cell walls of cobalt-resistant and sensitive mutants were observed. The physiological significance of distinct Ca and Mg binding sites on cell wall and the influence of toxic metal ions will be discussed.

554. Developmental Expression of Two Forms of Arginase from N. crassa. Gloria E. Turner and R. L. Weiss. University of California, Los Angeles, CA

The physiological role of multiple arginases in *N. crassa* is not understoood. The two forms are differentially expressed from a single locus (*aga*) and both proteins are localized to the cytoplasm. The 36-kD protein is expressed under all conditions, whereas the 41-kDa form is detected when *N. crassa* is grown in the presence of arginine. In this study we determine developmental expression of the two arginase transcripts and proteins by characterizing conidia and conidial germination at 2, 4 and 8 hours. Both major forms of the protein are stored in conidia, however the 1.4-kb transcript is not detected. The RNA's are temporally expressed during early germination. To better understand the role of arginase in conidia and the nature of the temporal expression we examined the effects of related metabolites; arginine, ornithine, proline, glutamate and glutamine on protein storage and temporal expression. These metabolites were used as supplements or sole nitrogen sources. The 1.4-kb transcript was never detected in conidial samples, however the 1.7-kb transcript was detected in conidia under all supplemented conditions. The temporal RNA expression early in germination, found in minimial arginine and ornithine supplementation, is abolished in proline, glutamate or glutamine supplementation as well as nitrogen limitation. In addition to abolishing the temporal expression, proline and glutamine repress aga transcription when they are the only source of nitrogen. Storage of arginase protein was detected in all conidial samples except when glutamate was used as the sole nitrogen source. These results support a novel pathway utilizing arginase for glutamate storage during conidiagenesis.

555. Characterization of four clustered genes associated with the biosynthesis of a red perithecial pigment in *Nectria haematococca*. Christelle Vasnier, Stéphane Graziani, Marie Dufresne and Marie-Josée Daboussi. Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France

Recently, we have identified a typical polyketide synthase (PKS) required for the synthesis of the red pigment present in the cell wall of perithecia of *Nectria haematococca*. Clustering between *pksN*, the gene encoding the PKS and other genes was expected in view of the typical genomic organization of genes involved in secondary metabolism pathways in fungi. Here we report the molecular organization of the genomic region surrounding *pksN* and the characterization of four novel genes clustered with *pksN*. The predicted amino acid sequences encoded by these genes designated *ppcA, ppcB, ppcC, ppcD* are very similar to those of cytochrome P-450 monooxygenase (*ppcA*), a putative NADH-flavin oxidoreductase (*ppcB*), an O-methyl transferase (*ppcC*) and an hypothetical protein displaying a DNA-binding domain typical of a GAL4-like Zn2Cys6 binuclear zinc finger (*ppcD*).
We performed disruption experiments to determine whether some of these genes are also involved in the red pigment biosynthesis. Disruption of *ppcA* led to total loss of the pigment suggesting that, as expected, it is part of a new biosynthetic gene cluster designated PP for perithecial pigment. Targeted disruption experiments of other genes are underway. Although sequences identical to the *AflR* binding sites were foundin the promoter regions of at least two of thefour ppc genes, it is not yet known whether the PP cluster contains an *AflR*-like regulatory gene.
Further work will allow us to determine the nature of the red pigment and will provide an interesting comparison of the biochemistry and biology between toxins and pigments in fungi.

556. Functional genomics studies for identifying genes involved in aflatoxin formation in *Aspergillus flavus*. Jiujiang Yu[1], Jeffery R. Wilkinson[1], H. Stanley Kim[2], William C. Nierman[2], Gary A. Payne[3], Joan W. Bennett[4], Jong H. Kim[5], Bruce C. Campbell[5], Deepak Bhatnagar[1], and Thomas E. Cleveland[1]. [1] USDA/ARS, Southern Regional Research Center, New Orleans, LA, USA. [2] The Institute for Genomic Research, Rockville, MA, USA. [3] North Carolina State University, Raleigh, NC, USA. [4] Tulane University, New Orleans, LA, USA. [5] USDA/ARS, Western Regional Research Center, Albany, CA, USA.

Aflatoxins are secondary metabolites produced mainly by the molds *Aspergillus flavus* and *A. parasiticus*. Because these compounds are toxic and extremely carcinogenic to animals, they pose a serious risk to human health. The aflatoxin biosynthetic pathway and its genetic regulation have been studied for decades, revealing a well organized aflatoxin pathway gene cluster consisting of 25 genes within a 70 kb DNA region. In order to better understand the molecular mechanisms that control or regulate aflatoxin production, identification of genes (gene profiling) using *A. flavus* expressed sequence tags (ESTs) and microarrays is currently being performed. Sequencing and annotation of *A. flavus* ESTs from a normalized *A. flavus* cDNA library identified 7,218 unique EST sequences. Genes that are putatively involved in aflatoxin biosynthesis, regulation and signal transduction, fungal virulence or pathogenicity, stress response or antioxidation, and fungal development were identified from these ESTs. Gene profiling using microarrays has thus far identified hundreds of genes that are highly expressed under aflatoxin-producing conditions. Further investigations on the functions of these genes are underway. This research is expected to provide information for developing new strategies for control of aflatoxin contamination of agricultural commodities.

Other

557. Molecular cloning of chitin synthase gene from *Rhizopus oryzae*. Ayumi Abe, *Yuji Oda, Teruo Sone, Kozo Asano . (Grad. Sch. Agr., Hokkaido Univ., Sapporo JAPAN *Natl. Agr. Res. Center Hokkaido, Memuro JAPAN)

Rhizopus oryzae and *Amylomyces rouxii* are well-known zygomycetes for fermentation foods in Asian countries They have the same rDNA ITS sequence and are a closely related to genome level. The remarkable difference between these two species is spore formation: *R. oryzae* makes abundant sporangiospore but less chlamydospore whereas *A. rouxii* rarely makes sporangiospore but makes abundant chlamydospore. In order to clarify this difference at molecular level, it is necessary to investigate the genes related to spore formation. In the recent research, the gene of class IV chitin synthase in *R. oligosporus* is revealed to be related to sporangiospore formation. Thus we analyzed chitin synthase genes in *R. oryzae*.
We tried to amplify chitin synthase genes (chs) from *R. oryzae* CBS 112.07 by using primer pairs for class I-III and class IV-V conserved amino acid sequences. Class I-III chs were divided into 6 clusters and all clusters indicated homology to class II conserved sequence• DClass IV-V chs were divided into 3 clusters and two clusters indicated homology to class IV and the other was class V. Southern hybridization analysis showed that *R. oryzae* had multiple chs genes in classes II and IV. Expression analysis revealed that one clsss II chs gene, named *chs3*, was expressed at spore formation and one class II chs gene, named *chs2*, was suppressed at spore formation. The *chs2* is 2726 bp, encoding 856 amino acids and contain 3 putative introns. The *chs3* is 2674 bp, encoding 852 amino acids and contains 2 putative introns.

558. Genetic analysis of cytoplasmic dynein structure and function in *Neurospora crassa*. Michael Plamann, David Madole, Dipti Gandhe, and Sonali Joshi. University of Missouri-Kansas City, School of Biological Sciences, Division of Cell Biology and Biophysics, Kansas City, MO 64110

Cytoplasmic dynein is a large, microtubule-associated motor complex that facilitates minus-end-directed transport of various cargoes. Dynein heavy chain (DHC) is >4000 residues in length, with the last two-thirds of the heavy chain forming the motor head. Six domains within the dynein motor exhibit varying degrees of homology to the AAA+ superfamily of ATPases. These domains are followed by a distinct C-terminal domain and together form a ring-like structure from which a microtubule-binding domain protrudes. Using a genetic assay in the filamentous fungus *Neurospora crassa*, we have isolated over 50 DHC mutants that produce full-length protein, but are defective in function. We have identified DHC point mutations in various areas including 1) AAA#1, the AAA module known to bind and hydrolyze ATP; 2) AAA#3, an AAA domain thought to regulate microtubule binding; 3) AAA#6, a domain with degenerate AAA homology lacking the conserved Walker boxes; and 4) AAA"#0", a globular domain with feint AAA homology which is N-terminal to AAA#1. In filamentous fungi such as *N. crassa*, cytoplasmic dynein is required for nuclear migration and retrograde vesicle transport of organelles. While all DHC point mutations examined so far are deficient in vesicle transport, some DHC point mutants exhibit apparently normal nuclear distribution phenotypes.

559. Reactive oxygen species regulate fungal cell differentiation. Karen Alvarez-Delfín, Nallely Cano, Teresa Lara-Ortíz, David Hewitt*, Wilhelm Hansberg and Jesús Aguirre. IFC, Universidad Nacional Autónoma de México and *Farlow Herbarium, Harvard University.

Reactive oxygen species (ROS) have been regarded as inevitable harmful by-products of aerobic metabolism. Growing evidence, however, suggests that ROS play important physiological roles. The phagocyte NADPH oxidase was the first example of an enzyme dedicated to ROS production. The catalytic component of this oxidase (NOX2) utilizes NADPH and O2 to generate O2•-. Novel members of the NOX family have been described in plants and animal non-phagocytic cells. In fungi, a phylogenetic analysis shows the presence of three new subfamilies (NOXA, B and C). The number of nox genes in fungi ranges from none in yeasts and dimorphic *U. maydis* and *C. neoformans* to one, two and three, revealing an interesting correlation between the presence of nox genes and the capability to develop multicellular fruiting bodies. In *A. nidulans*, ROS are generated during differentiation of cleistothecia. Inactivation of the NOX NoxA markedly reduces ROS production and blocks cleistothecia development at an early stage , while *noxA* derepression correlates with premature and abundant development of cleistothecia. Likewise, in *N. crassa* the inactivation of *nox-1* impairs fruiting body development, whereas the inactivation of *nox-2* prevents ascospore germination. Our results show that manipulation of reactive species, as strategy to regulate cell differentiation, is ubiquitous in eukaryotes.

560. Molecular karyotypes of the phytopathogenic fungus *Ascochyta rabiei* and related legume-infecting *Ascochyta* spp. Hajime Akamatsu and Tobin L. Peever. Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430, U.S.A.

The ascomycete fungus *Ascochyta rabiei* (Pass.) Labr. is the causal agent of Ascochyta blight of chickpea. Although it is possible to perform *in vitro* crosses with this fungus, electrophoretic karyotyping using pulsed-field gel electrophoresis (PFGE) has not been reported. PFGE was performed on a worldwide collection of *A. rabiei* and karyotypes were compared to other *Ascochyta* spp. including *A. fabae*, *A. lentis*, *A. pisi* and *A. viciae-villosae* from faba bean, lentil, pea, and hairy vetch, respectively. PFGE profiles of 45 isolates of *A. rabiei* from 19 countries revealed 10-14 chromosomes between 0.9 and 3.9 Mb and an estimated genome size of 20.5 to 30.5 Mb. Chromosome-length polymorphisms among chromosomes larger than 2.2 Mb were detected. PFGE of other *Ascochyta* spp. revealed a high level of interspecific variation in karyotype and all were highly dissimilar to *A. rabiei*. Numbers of chromosomes ranged from 13-17, 11-13 and 11-12 in *A. fabae*, *A. lentis* and *A. pisi* with sizes of 0.6 to 6.0, 0.6 to 4.6 and 0.4 to 3.8 Mb, respectively. The genome sizes of these species were estimated to be 30.4-39.5, 21.0-25.3 and 21.0-25.5 Mb, respectively. Assignment of nuclear ribosomal RNA and other genetic markers to chromosomes is being carried out by hybridizing cloned *A. rabiei* sequences to PFGE blots and linkage analyses. This data, combined with data from directed *in vitro* crosses, mating system analyses, host inoculations and phylogenetic analyses is being used to study the host specificity, genome evolution, and speciation of these fungi.

561. Human Bcl-2 genes affect vegetative growth, stress adaptation and apoptosis in the plant pathogen *Colletotrichum gloeoesporioides*. Sima Barhoom and Amir Sharon. Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel

Members of the evolutionarily conserved Bcl-2 family of proteins are central regulators of programmed cell death. They include apoptosis promoting (e.g. Bax, Bak) and inhibiting (e.g. Bcl-2, Bcl-xL) proteins, which regulate the mitochondria-mediated apoptosis pathway. In the present study we tested the ability of human Bcl-2 proteins to affect growth and survival in the plant pathogenic fungus *Colletotrichum gloeosporioides* f. sp. *Aeschynomene* (C.g.a.). Transgenic fungal isolates expressing the apoptosis-inhibiting Bcl-2 gene had various phenotypes including elevated levels of stress resistance, longevity, and modified spore and mycelium production. As a result, liquid cultures of the transgenic isolates had an extended logarithmic phase leading to over production of mycelium, enhanced conidia production in solid cultures, and higher tolerance to stresses. Transcriptional analysis revealed that the Bcl-2 isolates had elevated levels of stress resistance genes under non stress conditions. Furthermore, vegetative growth genes were found to retain high levels of expression under stress conditions. These and other results suggest that fungal vegetative growth and stress adaptation are tightly coordinated, and are regulated through a common mechanism which involves apoptosis-related proteins.

562. Adaptation of the TAP tag method to decipher protein complexes in filamentous fungi. Özgür Bayram, Kerstin Helmstaedt, Sven Krappmann and Gerhard H. Braus. Institute of Microbiology & Genetics, Georg-August-University Goettingen, Grisebachstr.8, 37077 Goettingen, Germany

Protein purification methods employing tandem affinity purification (TAP) tags have become an increasingly useful tool to gain information about the composition of cellular protein complexes and interactions among proteins. Generally, TAP tag constructs consist of two tandemly repeated Staphylococcus aureus protein A domains, one TEV protease cleavage site and a small peptide comprising a calmoduline binding domain. Originally designed for yeast expression, when expressed in the filamentous fungus Aspergillus nidulans detectable levels of TAP tag fusion proteins are rather low owing to the species' codon usage.

By application of site directed mutagenesis, we have altered all the rarely used codons in the commonly employed TAP tag construct to achieve higher rates of translation in the endogenous host. Both versions of the tag suited for N-terminal and C-terminal fusions were modified. Expression levels of these modified TAP tags were tested by construction of fusions to the green flourescent protein (GFP), the expression of which was driven by the inducible alcA promoter. After functionality could be validated, chimeric constructs with one regulator of A. nidulans fruit body formation were expressed and a purification protocol for complex enrichment could be established.

563. Quantitative trait locus (QTL) analysis in *Neurospora crassa* for discovery of new circadian clock genes. Cornelia Boesl[1], Elizabeth Turner[2], Elisabetta Trevellin[3], Martha Merrow[4], John Taylor[2] and Till Roenneberg[1]. [1]Ludwig-Maximilians-Uiversity, Munich, Germany; [2]University of California, Berkeley, USA; [3]University of Padua, Padua, Italy; [4]University of Groningen, Haren, The Netherlands.

We hypothesize that the *Neurospora* circadian clock is a multi-oscillator network that involves numerous genes and we are using a QTL approach to challenge our hypothesis. We measured free-running period (FRP) in constant darkness and the phase of entrainment (PoE) in a 12h/12h light-dark-cycle in each member of a QTL mapping population comprising 200 offspring of a cross between two wild type strains of *N. crassa*. Despite the fact that the parental strains have a similar FRP and phase, we found a wide distribution amongst the progeny for both FRP and PoE, consistent with the involvement of multiple genes in these traits. Contrary to circadian theory and experimental evidence, but similar to findings in *Arabidopsis*, we found no straightforward correlations between phase and FRP. The statistically strongest QTL that we identified was linked to the known clock genes, *frequency* and *white collar-1*, offering proof of principle.

564. Differentiation of onion neck rot *Botrytis* spp. in onion seed crops in Washington State and development of a real-time PCR assay for detection in onion seed. M.I. Chilvers[1], L.J. du Toit[2] and T.L. Peever[1]. [1]Washington State University, Department of Plant Pathology, PO Box 646430, Pullman, WA 99164-6430, [2]Washington State University - NWREC, 16650 State Route 536, Mount Vernon, WA 98273-4768.

Botrytis allii and *B. aclada* are the predominant species causing neck rot of onion, but are indistinguishable morphologically, with similar growth patterns on media and overlapping spore sizes. *Botrytis byssoidea* also causes neck rot but is far less prevalent and can be distinguished morphologically from *B. allii* and *B. aclada*. A collection of isolates of *Botrytis* spp. from the Columbia Basin of Washington State was examined using a PCR-RFLP technique developed by Nielsen et al (2002) for neck rot *Botrytis* spp. The primers specific to *Botrytis* spp. reported on onion were used to amplify the anonymous region of DNA of 469 isolates identified morphologically as *B. allii* or *B. aclada*, and digested with ApoI restriction endonuclease. The digested product was separated on agarose gel and stained with ethidium bromide. Sixty and forty percent of the isolates were identified as *B. aclada* and *B. allii*, respectively. The results demonstrate that both species are prevalent in onion seed crops in this semi-arid region of central Washington. A real-time fluorescent PCR assay was developed using SYBR green chemistry to quantify the amount of neck rot *Botrytis* spp. (*B. allii, B. aclada*, and *B. byssoidea*) present in onion seed. The nuclear ribosomal intergenic spacer (IGS) region of target and non-target *Botrytis* spp. was sequenced, aligned, and used to design primers specific to *B. allii, B. aclada*, and *B. byssoidea*. Primers and amplification parameters were optimized to avoid amplifying the related species *B. cinerea, B. porri, B. squamosa*, and *Sclerotinia sclerotiorum*, as well as 15 other fungal species commonly found on or in onion seed. The assay was capable of detecting 10 fg genomic DNA extracted from pure cultures of *B. allii* and *B. aclada*. Preliminary analyses demonstrate that the real-time PCR assay can be performed without inhibition of the PCR reactions by the seed extract.

565. Molecular and cellular mycology: using fungal models to illustrate advanced biological concepts to students.. Angus L. Dawe, Biology Department and Molecular Biology Program, New Mexico State University, Las Cruces, NM 88003

Traditional mycology courses have likely formed a part of the education of most of the attendees at the 23rd FGC. Such curricula provide essential insights into basic biology and phylogeny and often incorporate laboratory exercises that teach students the characteristics of different groups as they relate to fungal identification. However, in diverse departments that handle students in a wide variety of biological disciplines, basic fungal biology may not provide the challenges that more advanced students are seeking. Many courses that straddle the divide between upper-division undergraduates and graduate students explore advanced biological topics such as the detailed analyses of intracellular or extracellular communication, for instance, or comprehensive studies of pathways critical for development and differentiation. These examples are topics that are usually described in the context of high-profile model systems, and yet fungal molecular biologists are well placed to offer similar material using examples from fungal model systems. A new course recently developed in the Biology Department at New Mexico State University seeks to exploit the vast resources available from the fungal community in a syllabus that covers advanced topics in molecular and cellular biology, genetics and genomics. The syllabus content will be discussed, together with the issue of providing mycology-centered courses that will attract students from other biology disciplines.

566. An essential role for the *Neurospora frequency* gene in circadian entrainment to temperature cycles. Antonio M. Pregueiro[1], Nathan Price-Lloyd[2], Deborah Bell-Pedersen[3], Christian Heintzen[2], Jennifer J. Loros[1], Jay C. Dunlap[1].[1]Department of Genetics, Dartmouth Medical School, Hanover, NH 03755, [2]School of Biological Sciences, The University of Manchester, Manchester, M13 9PT, UK, [3]Department of Biology, Texas A&M University, College Station TX 77843

Circadian systems include slave oscillators and central pacemakers, and the cores of eukaryotic circadian clocks described to date are composed of transcription and translation feedback loops (TTFLs). In the model system Neurospora, normal circadian rhythmicity requires a TTFL in which a White Collar Complex (WCC) activates expression of the *frequency (frq)* gene, and the FRQ protein feeds back to attenuate that activation (reviewed in Dunlap and Loros, J. Biol. Rhythms 19, 414-424, 2004). To further test the centrality of this TTFL to the circadian mechanism in Neurospora, we exposed wild-type and *frq*-null strains to low-amplitude temperature cycles. We followed the peak in conidiation (a standard phase reference point) as well as the trough, and also novel (for analysis of Neurospora rhythms) phase reference points of "onset" and "offset" corresponding to the points where the daily cycle crosses the line represented by the running average level of conidiation. We found that the temperature cycle altered the waveform of the rhythm, especially the rate of rise to the peak, so that the "onset" reference point could not provide reliable estimates of phase among groups exposed to different period length temperature cycles. Based on all reliable reference markers, however, wild-type cultures were entrained to all the temperature cycles. Unlike normal strains, however, *frq*-null mutants did not truly entrain to the same cycles. Their peaks and troughs always occurred in the cold and warm periods respectively, strongly suggesting that the rhythm in Neurospora lacking *frq* function is driven by the temperature cycles. Altogether, these data as well as results of additional tests indicate that the FLO, at best, reflects the output of a weak oscillator that is a slave to the TTFL which underlies circadian rhythm generation in Neurospora.

567. A simplified method for collecting secreted proteins from *Botrytis cinerea*. H. El Mubarek, G.K. Podila and M.R. Davis. University of Alabama in Huntsville, Huntsville, AL.

Botrytis cinerea is an economically important plant pathogenic fungus with a broad plant host range. Conidia of *B. cinerea* loosely attach to the substrata by hydrophobic interactions that are easily interrupted. Upon germination, the previous interactions are superseded by the secretion of an extracellular matrix (ECM). The ECM is extremely resistant to removal from various strata and suggested to be important for the infection process. In liquid culture, the ECM has also been found to trap many of the organism's secreted proteins and masks their activities. We describe a method that anchors the fungus to cellophane or nylon membranes that are also permeable to the secreted protein. This anchoring of the fungus to a membrane reduces the deposition of the ECM, which may be excessively released as the organism searches for a substratum in a liquid culture. The pool of enzymes secreted from *B. cinerea* is measurably increased and can be used for subsequent purification and protein characterization. Similarly, this method allows for isolation of ECM from *B. cinerea*, away from secreted proteins, for further characterization.

568. NpkA, a cdc2-related kinase from *Aspergillus nidulans*, interacts with the UvsB^{ATR} kinase. Fagundes, Mrvzk[1], Savoldi, M[1], Lima, JF[1], Malavazi, I[1], Larson, RE[2], Goldman, MH[3]; Goldman, GH[1]. [1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto; [2]Faculdade de Medicina de Ribeirão Preto; [3] Faculdade de Filosofia Ciências e Letras de Ribeirão Preto,USP, Brazil.

The DNA damage response is a protective mechanism that ensures the maintenance of genomic integrity. We have been using *Aspergillus nidulans* as a model system to characterize the DNA damage response caused by the anti-topoisomerase I drug, camptothecin (CPT). We report the molecular characterization of a p34Cdc2-related gene, *npkA*, that is transcriptionally induced by CPT and other DNA-damaging agents, and its induction in the presence of CPT is dependent on the *uvsB*^{ATR} gene. The *npkA* deleted strain can partially suppress HU-sensitivity caused by the *uvsB*^{ATR} and *uvsD153*^{ATRIP} checkpoint mutations. We demonstrated that *uvsB*^{ATR} gene is involved in DNA replication and the intra-S-phase checkpoints, and that the *npkA* deleted strain can suppress its intra-S-phase checkpoint deficiency. There is a defect in both the intra-S-phase and DNA replication checkpoints due to the *npkA* inactivation when DNA replication is slowed at 6 mM HU. Our results suggest that *npkA* gene plays a role in cell cycle progression during S phase as well as in a DNA-damage signal transduction pathway in *A. nidulans*.
Financial Support: FAPESP e CNPq

569. Amplification of fungal genomes by multiple displacement amplification. Simon J Foster and Brendon J Monahan. Centre for Functional Genomics, Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand.

The availability of genomic DNA (gDNA) of sufficient quality and quantity is fundamental to molecular genetic analysis. With the increasing amount of genome sequence data available for filamentous fungi, high-throughput methods for gDNA isolation are required, predominantly for rapid screening of deletion mutants. Many fungi are slow growing or even unculturable (e.g. obligate pathogens of plant or animals) and current DNA isolation methods for these fungi are often unsatisfactory, giving low yields and poor quality DNA. We present here the use of a whole genome amplification method for the rapid production of fungal genomic DNA.
Multiple displacement amplification (MDA) using *phi29* DNA polymerase was used to amplify whole genomes for two fungal species, *Penicillium paxilli* and the slow growing endophyte of grasses *Epichloë festucae*. Up to 10 micrograms of high molecular weight DNA was routinely amplified from less than 10 ng of crude template DNA. Rapid methods used to obtain template DNA for MDA reactions were optimized and differed for the two fungi. For *P. paxilli*, which sporulates profusely, mechanical disruption of spores using glass beads was sufficient; for the non-sporulating endophyte, alkaline lysis of mycelium was required.
The use of MDA-derived gDNA for downstream applications was assessed. PCR of target sequences up to 10 kb was possible and the DNA could be digested using restriction enzymes. Southern blot analysis was successful, although results depended on the size of hybridizing bands. We also show, by hybridisation to a cosmid library, that MDA-amplified DNA is representative of the genome.

570. *Agrobacterium tumefaciens* -mediated genetic transformation of *Aspergillus carbonarius*. Maria Helena P. Fungaro, Luiz Rodrigo Ito, Rubens Tadeu Duarte, Márcia Cristina Furlaneto. Centro de Ciências Biológicas, Universidade Estadual de Londrina, CP, 6001, CEP 86051-990, Londrina-Paraná. Brazil. *Corresponding author

Aspergillus carbonarius is a potent ochratoxin A (OTA) producer that has been found in cereal and food commodities. OTA has nephrotoxic effect and it has been classified as a possible carcinogenic substance for humans. Genetic studies of *Aspergillus carbonarius* are poorly developed and here we described a genetic transformation system for this filamentous fungus, providing an important step towards the genetic manipulation of this ochratoxigenic species. The fungus was transformed to hygromycin B resistance using the AGL-1 strain of *Agrobacterium tumefaciens* harboring the binary vector pPK2. Transformation frequencies ranged from 23 to 101 transformants per 10^5 conidia. Highly resistant transformants were obtained which showed capacity of growing on increased concentrations of hygromycin B (up to 1200 ug ml^{-1}).High mitotic stability of the transformants (94%) was demonstrated after ten successive transfers on non-selective media.Considering the efficiency and flexibility of *A. tumefaciens*-mediated transformation, this method appears to be an efficient alternative to other insertional mutagenesis techniques in characterizing genes related to ochratoxin biosynthesis.
Acknowledgements CAPES/CNPq/Fundação Araucária/CPG-UEL

571. *Agrobacterium tumefaciens*-mediated transformation and protease mutants isolation in the entomopathogenic fungus *Metarhizium anisopliae* var *acridum*. Marcia Cristina Furlaneto[1], Rubens Tadeu Delgado Duarte[1], Ariane Coelho Donatti[1], Celso Vataru Nakamura[2], Augusto Schrank[3], and Maria Helena Pelegrinelli Fungaro[1]. [1]Universidade Estadual de Londrina, Microbiologia, Londrina, PR. [2]Universidade Estadual de Maringá, Análises Clínicas, Maringá-PR. [3]Universidade Federal do Rio Grande do Sul, Centro de Biotecnologia, Porto Alegre-RS, Brazil.

Metarhizium anisopliae var. *acridum* is regarded as one of the most promising species in the development of practical insect biological control agents of grasshoppers In Brazil, a biocontrol program has been assembled for the purpose of developing a native strain (CG423) of *M. anisopliae* var. *acridum* as a bioinsecticide against the grasshopper *Rhammatocerus schistocercoides* which causes severe agricultural losses. We report for the first time that *Agrobacterium tumefaciens* strain AGL-1 attaches to and genetically transforms *M. anisopliae* var. *acridum* strain CG423. The *Agrobacterium*-mediated transformation was applied using two distinct binary vectors carrying a benomyl resistance (b-tubulin) gene as a selection marker. The efficiency of transformation was up to 53 transformants per 10^5 target conidia. High mitotic stability of the transformants (89-95%) was demonstrated after five successive transfers on non-selective media. Highly resistant transformants were obtained which showed capacity of growing on increased concentrations of benomyl (up to 100 ug ml^{-1}). We obtained three putative T-DNA-tagged mutants with altered protease production. Thus, the described protocol could provide a useful tool to tag genes that may be important for pathogenesis and virulence of this fungus.
Acknowledgements: CNPq/CAPES/CPG-UEL

572. Transformation of the entomopathogenic fungus *Paecilomyces fumosoroseus* using T-DNA from *Agrobacterium tumefaciens* . Marcia Cristina Furlaneto, Ivan Gláucio Paulino Lima, Rubens Tadeu Delgado Duarte, Luciana Furlaneto, and Maria Helena Pelegrinelli Fungaro. Universidade de Londrina, Microbiologia, Londrina, Parana – Brazil.

Paecilomyces fumosoroseus causes diseases in the whitefly *Bemisia tabaci* which is responsable for damage to a variety of high value crops worldwide. *Agrobacterium tumefaciens*-mediated transformation was successfully applied to *P. fumosoroseus*. Conidia of *P. fumosoroseus* were transformed to hygromycin B resistance using the *hph* gene of *Escherichia coli* as the selective trait, under the control of a heterologous gpd promoter and trpC terminator from *Aspergillus nidulans*. The efficiency of transformation was up to 74, 127 and 247 transformants per 10^5, 10^6 and 10^7 target conidia, respectively. Following 5 serial passages of transformants on non-selective medium, 100% of the transformants were found to be mitotically stable by a conidial germination test. *A.tumefaciens*-mediated transformation yielded stable transformants capable of growing on increased concentrations of hygromycin B (up to 900 ug/ml).The presence of *hph* gene was confirmed by PCR. Southern analysis revealed that the *P. fumosoroseus* transformants contained a randomly integrated single ou multiple T-DNA copies in the genome. Inverse PCR will be used to recover the *P. fumosoroseus* DNA flanking the T-DNA. Considering the efficiency and flexibility of *A. tumefaciens*-mediated transformation, this method appears to be an efficient alternative to other insertional mutagenesis techniques in characterizing genes that are important for the pathogenicity of *P. fumosoroseus*.
Acknowledgements: CNPq/CAPES/CPG-UEL

573. Analysis of *MAT* ideomorphs in *Colletotrichum lindemuthianum*. Garcia-Serrano, Monica[1], Rodriguez-Guerra, Raul[2] and Simpson June[1]. 1. Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, Unidad Irapuato, Irapuato, Gto., México. 2. INIFAP, Unidad Celaya, Celaya, Gto., México.

Colletotrichum lindemuthianum causes anthracnose on common bean *(P. vulgaris)* and Under favorable conditions can cause up to 100% losses in bean production. Although classified as a Deuteromycete, sporadic reports have shown that under laboratory conditions *Colletotrichum lindemuthianum* can be made to undergo a sexual reproductive cycle.
We have isolated two Mexican strains of *C. lindemuthianum* capable of reproducing sexually and our interest is to study mating in this species and compare this process with a closely related species *G. gloesporoides (G. cingulata)* which freely undergoes sexual reproduction in nature.
Degenerate oligonucleotide primers specific for the MAT 1-2 locus of ascomycetes produce fragments in both parental strains as is the case for other plant pathogens such as *C. Graminicola* and *B. sacchari* .
We are currently characterizing the MAT 1-2 ideomorphs of the parental *C. lindemuthianum* strains by TAIL-PCR and the construction of mini-genomic libraries. This characterization will be presented and discussed in relation to other ascomycetes.

574. The role of faculty-graduate collaborative course design in teaching scientific reasoning to undergraduates. Caleb A. Hodson [1] and Patricia J. Pukkila [2], [1]Graduate Program in Cell and Molecular Physiology and [2] Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Effective undergraduate science education must include opportunities for students to engage in original scientific inquiry. We have found that a faculty-graduate student team is ideally suited to design and implement a sophomore-level seminar ("Introduction to Research in Biology") in which students learn to interpret scientific conclusions as the products of research, to generate and evaluate original ideas, and to develop proposals for their own work. Throughout the course, the instructors focus both on the elements of scientific reasoning (particularly the evaluation of scientific claims) and also on the attitudes and values of practicing scientists (especially the need to convince one's peers of the validity of one's arguments). These two elements allow the undergraduates to apply new habits of mind to questions of interest to them during the seminar and to other courses in both scientific and non-scientific areas both during the semester and after. The collaborative design and implementation of the course enhances its effectiveness and contributes positively to graduate professional development. We conclude that our model is adaptable to a wide range of disciplines and allows faculty, graduate and undergraduate students to share in the stimulating intellectual environment offered by a research university.

575. Recombination is enhanced in regions flanking the *Cryptococcus* mating type locus: implications for the evolution of a fungal sex chromosome. Yen-Ping Hsueh[1], Alex Idnurm[1], and Joseph Heitman[1]. [1]Department of Molecular Genetics and Microbiology, Duke University, Durham, NC

Meiotic recombination is unevenly distributed throughout the genome. As a consequence, genetic and physical map distances do not have a simple linear relationship. Recombination hot spots and cold spots have been described in many fungi and recombination rates may in part reflect global features of chromosome structure. Here, we report that recombination is elevated adjacent to the mating type locus (*MAT*) in the pathogenic basidiomycete *Cryptococcus neoformans*. *C. neoformans* has an unusually large *MAT* locus, and recombination is suppressed between the alleles of this sexually dimorphic region of the genome. By introducing genetic markers at defined physical distances on both sides of *MAT*, we scored the meiotic recombination frequency on the *MAT* chromosome. The frequency of meiotic recombination is ~20% between the *MAT* locus and a flanking marker at 5, 10, 50 or 100 kb from the right or left border of *MAT*. This is approximately ten times higher than the recombination frequency between the markers at 10 and 100 kb from the *MAT* right border. Moreover, we found that recombination frequently occurs on both the left and the right sides of *MAT*. This result suggests that during meiosis, *C. neoformans* may exchange the *MAT* locus onto different genetic backgrounds and meanwhile, restrict the *MAT* locus from expanding to prevent capture of the entire chromosome. *MAT* linked recombinational enhancers may also have facilitated capture of genes into *MAT* by enabling rapid assimilation into both mating type alleles. We are currently investigating whether recombinational enhancers flank *MAT* or reside in the **a** or alpha *MAT* allele, and testing if *MAT* heterozygosity is required to stimulate recombination.

576. Growth defect and mutator phenotypes of RecQ-deficient *Neurospora crassa* mutants. Akihiro Kato and Hirokazu Inoue (Saitama University, Japan).

RecQ helicases function in the maintenance of genome stability in many organisms. The filamentous fungus *Neurospora crassa* has two RecQ homologues, QDE3 and RECQ2. We found that the *qde-3 recQ2* double mutant showed a severe growth defect. In addition, sequences introduced by transfection integrated homologously much more frequently in the *qde-3 recQ2* double mutant than in the wild type, in which homologous integration is rare. The growth defect was alleviated and the increase in homologous integration suppressed by mutation in *mei-3*, the homologue of yeast *RAD51*, which is required for homologous recombination (HR). These results suggest that HR is responsible for both phenotypes.

577. Infecting an introductory bioinformatics course with your favorite fungus. Steve James. Biology Department, Gettysburg College, Gettysburg, PA

Bioinformatic tools are indispensable in the daily life and work of molecular biologists. Putting these powerful tools into the hands of aspiring undergraduates provides a rigorous intellectual challenge that fosters development of original thinking and problem-solving. Fungal genomes furnish an ideal training ground for *in silico* gene discovery and analysis, owing to their high gene density, the relative diminution of introns, and the increasing availability and variety of fungal genomes. In a new bioinformatics course at Gettysburg College, students undertake a comprehensive project to annotate 50,000 bp of a recently released, pre-annotation fungal sequence. In the inaugural course offering, during fall of 2004, students studied the *Coccidioides immitis* genome. Using NCBI tools such as ORFinder, tblastn, and blastp, students identified *C. immitis* genes, learned to judge the quality of hits, merge exons, recognize conserved domains, and predict orthologs. The students identified paralogs within *C. immitis*, and constructed phylogenies among paralogs and orthologs using ClustalW. In addition, students examined synteny between the closely related *C. immitis* and *A. nidulans* genomes. Students also employed the Saccharomyces Genome Database (SGD) to investigate expression profiles and protein-protein interactions of the yeast homologs of *C. immitis* genes. An example of a web-based final project will be presented to show the breadth and quality of learning that can be achieved by this type of project-based approach.

578. Molecular mechanisms of stress response in the arbuscular mycorrhizal fungus *Gigaspora margarita*. Luisa Lanfranco and Paola Bonfante. Dipartimento Biologia Vegetale, Università di Torino and Istituto Protezione Piante, C.N.R., Torino, Italy.

Arbuscular mycorrhizal (AM) fungi form a highly compatible root symbiosis. In natural and agricultural environments, they significantly contribute to plant growth by improving mineral nutrition and by protecting plants against a variety of biotic and abiotic stresses. Little information is currently available on the molecular mechanisms of stress response operating in these organisms and whether they contribute to the dialogue with the host plant.
An EST collection from germinated spores of the AM fungus *Gigaspora margarita* (BEG 34) turned out to be a valuable source of genes related to defence responses. A gene encoding a metallothionein was characterised (Lanfranco et al., 2002, Plant Physiol). By screening a genomic library the promoter region was isolated and its transcriptional activity is currently under investigation. Another clone showed high similarity to CuZn superoxide dismutases (SODs). This gene was differentially expressed during the fungal life cycle. In two different host plants the highest transcript levels were found in the intraradical fungal structures. These structures were also positive to DAB reaction, used to detect H_2O_2 accumulation. These results suggest that fungal ROS scavenging systems may be an unexpected component of the plant/fungus dialogue, which allows a functional compatibility between the partners. Investigations on whether the gene is modulated upon exposure to different stresses are under way.

579. The alignment between physical and genetic maps of *Gibberella zeae* J.Lee(1), J.E. Jurgenson(2). J.F. Leslie(1) and R.L. Bowden(3). (1) Kansas State University, Manhattan,KS; (2) University of Northern Iowa, Cedar Falls, IA; (3) USDA-ARS Plant Science and Entomology Research Unit, Manhattan, KS.

Jurgenson et al. (2002) previously published a genetic map of *Gibberella zeae* (*Fusarium graminearum*) based on a cross between Kansas strain Z-3639 (lineage 7) and Japanese strain R-5470 (lineage 6). The genetic map was based on 1048 AFLP markers and consisted of nine linkage groups. We aligned the genetic map with the first assembly of the genomic sequence of strain PH-1 (lineage 7) that was released by The Broad Institute (Cambridge, MA). We used 7 sequenced structural genes and 129 sequenced AFLP markers from all nine linkage groups (LG) of the genetic map. One hundred and fourteen markers were associated with nine supercontigs (SC) of the genomic sequence. LG1, LG7, LG8 and LG9 aligned with SC2 and SC5; LG2 aligned with SC3, SC8 and SC9; LG 3 aligned with SC4 and SC6; and LG4, LG5 and LG6 aligned with SC1 and SC7. Approximately 99% of the sequence was anchored to the genetic map, indicating the high quality of the sequence assembly and the relative completeness and validity of the genetic map. The alignments grouped the linkage groups and supercontigs into four sets, suggesting that there are four chromosomes in this fungus.

580. Interspecific Interactions between *Ustilago maydis* and fungal endophytes of maize. Keunsub Lee[1], Jean Pan[2], Georgiana May[1,2]. University of Minnesota [1]Plant Biological Sciences Graduate Program. [2]Department of Ecology, Evolution & Behavior, Minnesota, United States

Ustilago maydis, a basidiomycete fungus, can infect any above ground tissues causing smut disease in maize and teosinte. We isolated and identified a number of maize endophytes. These are asymptomatic in terms of disease symptoms but endophytes are known to have other effects against other pathogens and pests. However, the interactions between endophytes and Ustilago maydis are unknown. As a first step to investigate interspecific interactions between Ustilago maydis and endophytes, we identified Fusarium species - the most common fungal endophytes in maize - using the TEF-1 alpha sequence. Among 82 isolates, we identified 22 F. sporotrichioides, 20 F. verticillioides, 15 F. graminearum, 15 F. subglutinans, 6 F.proliferatum, 1 F.cerealis, 1 F. poae, and 1 F. oxysporum. We examined their competitive interactions in vitro.

581. The *Coprinus cinereus* Genome Project in the Teaching Laboratory. Walt Lilly and Allen Gathman, Southeast MO State Univ., Cape Girardeau, MO

To be well prepared for careers in research, students need significant, inquiry-based laboratory activities. For several years we have offered an inquiry-based molecular biology laboratory course, Investigative Molecular Biology and Biotechnology (IMBB), which focuses on individual student investigations. We have recently integrated materials and objectives from the *C. cinereus* Genome Project into IMBB. Students isolate random clones from cDNA libraries, sequence them, determine insert size, and assess the presence of their clones in the genomes of *C. cinereus* and related basidiomycetes using Southern hybridization. Students also perform bioinformatics analyses on the isolated gene sequences and their predicted products, including mapping them to the genomic sequence using GBrowse. Ultimately, each student prepares a publication-style manuscript. The challenges of this approach include designing research around pre-arranged lab time, keeping students focused on the semester-long objective, the cost per student, and the reality that experiments don't always proceed as expected. Technical problems of failed PCR reactions, poor quality genomic DNA isolations, and poor student lab skills present a failure risk. Students also face the reality of our limited knowledge of basidiomycete genes. In fall 2004, students selected 18 clones from a cDNA library. Nine of 14 successful sequences failed to match any known genes using Blastx. Despite the challenges, we find that student understanding of and interest in research are improved by this teaching model.

582. A mitochondrial short-chain acyl-CoA dehydrogenase from *Aspergillus nidulans*. Lori A. Maggio-Hall and Nancy P. Keller. Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin, USA

We have recently described a mitochondrial fatty acid beta-oxidation pathway in *Aspergillus nidulans* (Maggio-Hall & Keller. 2004. *Mol. Microbiol.* 54: 1173-85). Here we describe the disruption and characterization of another gene in the pathway, that encoding the acyl-CoA dehydrogenase (*scdA*). The phenotypes of the *scdA* deletion strain further confirmed this pathway's role in degrading short-chain fatty acids (C4-C6) as well as the amino acids isoleucine and valine. Unlike the previously described disruption of the enoyl-CoA hydratase enzyme in the pathway, the *scdA* mutant showed no defect during growth on long- and very long-chain fatty acids. Furthermore, the *scdA echA* double mutant also grew just as well as wild type on these fatty acids, indicating that the *echA* deletion phenotype—extremely restricted growth on long- and very long-chain fatty acids—was due entirely to toxicity of accumulated intermediates and not a failure to metabolize the fatty acids. Biochemical analysis of the *scdA* (dehydrogenase) mutant 12 h after transfer to hexanoate-containing medium showed that whole-cell enoyl-CoA hydratase activity was significantly diminished, suggesting a build-up of short-chain fatty acids inhibits other beta-oxidation pathways in the cell. This inhibition is not at the level of transcription, based on Northern analysis of identified enoyl-CoA hydratase-encoding genes (*echA* and *foxA*).

583. *Aspergillus nidulans rad50* gene inactivation leads to hypersensitivity to DNA damaging agents, and sexual and cell cycle checkpoint defects. Iran Malavazi[1], Joel Fernandes Lima[1], Márcia Regina von Zeska Kress Fagundes[1], Vladimir P. Efimov[2], Maria Helena de Souza Goldman[3] and Gustavo Henrique Goldman[1]. [1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto and [3]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil. [2]Department of Pharmacology, University of Medicine and Dentistry of New Jersey, USA.

The hMRE11-hRAD50-NBS1 protein complex has emerged as a central player in the human cellular DNA damage response, and recent observations suggest that these proteins are at least partially responsible for the linking of DNA-damage detection to DNA-repair and cell cycle-checkpoint functions. We have identified *A. nidulans rad50* mutant in a screen for dynein synthetic lethals. The *rad50* gene was cloned by complementation of the sporulation deficiency phenotype of this mutant. A transversion G-C at the position 2509 (Ala-692-Pro amino acid change) in the *rad50* mutant causes sensitivity to several DNA damaging agents. This mutation is novel and occurs at the coiled-coil region of the Rad50. We have deleted part of the coiled-coil region and few aminoacids of the Rad50-Mre11 interaction region and assessed several phenotypic traits in this deletion strain. Besides sensitivity to several DNA damaging agents, this deletion strain is also impaired in the intra-S-phase and DNA replication checkpoints. There is a genetic interaction between *rad50* and $bimE^{APC1}$ at both S-phase checkpoints. Interestingly, we have also seen epistasis between *rad50* and $uvsB^{ATR}$ when germinating conidia of the corresponding double mutant are exposed to ultraviolet light. In addition, the *rad50* gene is also important for ascospore viability.
Financial support: FAPESP and CNPq, Brazil

584. Diversity of fungal-like protists cultured from marine invertebrate guts. Wyth L. Marshall and Mary L. Berbee. University of British Columbia.

Zoosporic fungi, an assemblage of poorly understood aquatic eukaryotes, are both effective parasites and primary decomposers in aquatic ecosystems. Some species are also known to proliferate inside the guts of marine invertebrates with apparently benign, possibly mutualistic, effects. There are three lineages of these micro-eukaryotes: the Labyrinthulomycota and Oomycota (Stramenopiles), the Chytridiomycota (basal fungi), and the Mesomycetozoans (a newly discovered clade near the animal-fungal divergence). Despite their differences in origin, these groups are strikingly similar in many aspects of their reproductive, colonization, and nutritional strategies -- in effect occupying very similar ecological niches. Traditional fungal culturing techniques are being used to investigate the diversity of these fungal or fungal-like microbes within marine invertebrate digestive tracts. Host species have included echinoderms, bivalves, sipunculids, tunicates and polychaetes. As expected, one or more distinct thraustochytrid species (based on sequence identity) can be cultured from each animal, however, Mesomycetozoans are also present in many of these cultures. Sequence data from the 18S rDNA from the Mesomycetozoan isolates show 95-98% similarity to Pseudoperkinsus tapetis and Spaeroforma actica. Within this class less than ten genera have been described, all have been isolated from animals, and most are intractable to culture. The discovery of these new isolates suggests that the biodiversity and prevalence of this class is much higher.

585. Raft domains in AM fungi? Aurora Ocon and Natalia Requena. University of Tuebingen, Physiological Ecology, Tuebingen, Germany

Glomus intraradices GiGIN1 gene is the orthologue of a novel gene, *GmGIN1*, found in *Glomus mosseae*, only expressed during early developmental stages of the life cycle of the arbuscular mycorrhizal fungus. The predicted GiGin1 protein, have a two-domain structure with a putative self-splicing activity. The N-terminal domain shares sequence similarity with a novel family of GTP binging proteins while the C-terminus has a striking homology to the C-terminal part of hedgehog protein family from metazoa. The C-terminal part of hedgehog proteins is known to participate in the covalent modification of the N-terminus by cholesterol, and in the self-splicing activity which renders the active form of the protein with signalling function. Raft domains are microdomains at the plasma membrane enriched in the cholesterol and sphingolipids. They serve as platform for proteins to interact with each other during signalling events. Hh-Np, the mature signalling form of hedgehog proteins, has been shown to partition into the rafts due to its cholesterol moiety. We speculate that the active N-terminal domain from Gin1 also localizes to the plasma membrane, most likely at the raft domains, where presumably participates in the signalling events prior to symbiosis formation. To prove this hypothesis, we have first investigated the presence of lipid rafts in *G. intraradices*. They have been purified from spores and hyphae using Triton X-100. The presence of Gin1 in lipid rafts is being analyzed by western blot using specific antibodies. The plasma membrane H+ATPase, a raft domain marker, is being used as a positive control. The protein content of the rafts will be analyzed by 2D-gel electrophoresis and mass spectrometry.

586. Rapid RNA expression profiling of the fungus *Trichoderma reesei* in production conditions. Jari Rautio,[1,2] Kari Kataja,[1] Reetta Satokari,[1] Merja Penttilä,[1] Hans Söderlund,[1] and Markku Saloheimo[1]. [1]VTT Biotechnology, PO Box 1500, FIN-02044 VTT, Finland; [2]Bioprocess Engineering Laboratory, Department of Process and Environmental Engineering, University of Oulu, Finland

Microorganisms used for various types of bioprocesses are subjected to constantly changing environmental conditions to which they adapt by adjusting their cellular physiology. Changes in the genomic expression program are the first signs of adaptation to chancing conditions or to potential process disturbances. However, tools suitable for high-throughput expression monitoring of process-relevant genes are scarce. Moving to postgenomic era with a growing number of organisms has increased the interest in functional genomics, and the need for fast and reliable transcriptional profiling methods is thus growing. We have developed a rapid method for transcriptional profiling of microbial cultivations based on a novel technique called TRAC "transcriptional profiling with the aid of affinity capture". This method allows fast gene expression analysis for sets of mRNAs by solution hybridisation with a pool of target-specific oligonucleotide probes of distinct sizes that are identified and quantified by capillary electrophoresis. The assay procedure has been semi-automated for simultaneous treatment of 96 samples using magnetic bead particle processor. To further enhance the robustness of the method it was set up to work with crude cell lysates. TRAC has been shown to produce results highly consistent with mRNA quantification by Northern hybridisation. Computational methods have been applied for design of target-specific oligonucleotide probes and to assign them into minimal number of pools. The whole assay procedure can be performed in three hours, implying its usefulness in bioprocess monitoring and control.
The developed TRAC method application has been used for monitoring the levels of a set of mRNAs in the filamentous fungus *Trichoderma reesei* in fermentation conditions. Chosen gene markers for bioprocess monitoring are involved in various cellular pathways including unfolded protein response, protection against various stress conditions, oxygen and nutrient limitation responses, protein synthesis and growth. Data collected from different types of fermentations, batch, fed-batch and continuous cultures, shows the potential of the method for use in optimisation of production processes and provides novel information about regulation of various genes during different phases of long cultivations. The TRAC method has also been used successfully for assessment of the steady states in chemostat cultures.

587. *Agrobacterium tumefaciens*-mediated transformation of the arbuscular mycorrhizal fungus *Glomus intraradices*. Margret Ecke, Reinhard Fischer and Natalia Requena. University of Tubingen, Plant Ecophysiology, Tubingen, Germany

Genetic manipulation of arbuscular mycorrhizal (AM) fungi has been hindered by their obligate biotrophism that narrows the cultivation under axenic conditions. In order to establish a stable transformation methodology for these fungi, we took advantage of the *Agrobacterium tumefaciens* mediated transformation and of an in vitro mycorrhizal system where large amounts of extraradical mycelium can be produced under axenic conditions. We carried out sensitivity screenings to different fungicides (hygromycin, benomyl and phosphinothricin) to be used as positive selection markers in this system. We found that hygromycin and benomyl were very effective at low concentrations (25 and 0.01 ?g/ml respectively) against both spore germination and hyphal growth of *G. intraradices* in contrast to the herbicide phosphinotricin. A binary vector was constructed containing the GFP reporter gene and hygromycin as selection marker, both under the control of the heterologous gpd promoter from *Aspergillus nidulans*. Extraradical hyphae of *G. intraradices* were transformed with this construct by co-cultivation with *A. tumefaciens*. Fluorescence microscopy showed a high number of transformed AM hyphae expressing GFP fluorescence. This is the first report of a mycorrhizal fungus where GFP fluorenscence has been observed and it shows that the *Agrobacterium*-mediated transformation could be further developed for functional analysis of fungal genes in the AM symbiosis.

588. Biochemical chacterization of the self-splicing protein GIN1 from the arbuscular mycorrhizal fungus Glomus mosseae. Natalia Requena and Esther Serrano. University of Tubingen, Plant Ecophysiology, Tubingen, Germany

GmGin1 is a mycorrhizal two-domain protein expressed during early developmental stages in *G. mosseae* . The C-terminus has a striking homology to the C-terminal part of the hedgehog protein family from metazoa. Hedgehog proteins are able to undergo self-splicing thanks to the Hint domain located in their C-terminus. As a result the mature N-terminal part is released covalently modified by a cholesterol moiety at its carboxy end. This lipid modification determines the proper localization of the protein at the cell membrane. We hypothesized that GIN1 would be also able to undergo self-splicing due to the conservation of the Hint domain. To assess this hypothesis the C-terminal domain was His-tagged purified. Gin1-C was shown to undergo splicing by addition of small nucleophiles such as DTT. To determine which molecule provokes the splicing *in vivo*, splicing assays with cholesterol and lipid extracts from spores, external mycelium and mycorrhized roots of *G. intraradices* were performed. The N-terminus of GIN1 shares similarity with the GTP binding protein family (IAN) evolutionary conserved from plants to humans. They are related to the control of local host defense against pathogens. We have expressed a His-tag Gin1-C protein in order to perform affinity and hydrolysis assays with radiolabelled nucleotides using cdc42 from *Ustilago maydis* as a positive control. Our hypothesis is that GmGIN1-N covalently bound to cholesterol will localize at a cell membrane and have GTPase signaling activity.

589. Truncation of the C-terminal domain of the ammonium transporter Mep2 abolishes transport but spares ligand sensing during *Saccharomyces cerevisiae* pseudohyphal growth. Julian Rutherford, John Rohde, Sandra Vergara and Joe Heitman. Molecular Genetics and Microbiology, Duke University, Research Drive, Box 3546, Durham, NC 27710 USA.

Diploid cells of the yeast *Saccharomyces cerevisiae* undergo pseudohyphal growth when both a fermentable carbon source is present and nitrogen is limiting. Previous studies indicate that this developmental process is dependent on the ammonium transporter, Mep2, which functions to both transport and sense ammonium ions. Our aim is to understand how Mep2 is able to sense ammonium levels that are low but sufficient to support a dimorphic switch. We have identified a novel *MEP2* allele, *MEP2-T*, which encodes a truncated Mep2 that lacks the C-terminal cytoplasmic domain. Expression of the *MEP2-T* allele results in increased levels of pseudohyphal growth in cells that contain the wild-type *MEP2* allele. However, the Mep2-T mutant fails to restore filamentous growth in a *mep2/mep2* mutant. Together, these data establish that the C-terminus of Mep2 has a regulatory function and that Mep2 and the Mep2-T mutant function co-ordinately to signal. In addition, the Mep2-T mutant is unable to transport ammonium, indicating that the transport and sensing functions of Mep2 are separate. Interactions of the C-terminal domain with other cytoplasmic domains of Mep2 and other proteins is under investigation.

590. The distribution of chronotypes in *Neurospora crassa*. Shahana Sultana, Till Roenneberg, David Jacobson§ and Martha Merrow. Institute for Medical Psychology, University of Munich, Munich, Germany; §Stanford University, CA, USA; *Biologisch Centrum, University of Groningen, Haren, The Netherlands.

Circadian clocks in all phyla control functions from activity and wakefulness to gene expression. Individual clocks synchronize (entrain) differently to the 24-hour day, creating different 'chronotypes'. A chronotype is determined by the interaction of the circadian clock with environmental factors, such as light. Chronotype has been used as a quantitative tool to determine the complex genetic basis of the circadian clock. It can be quantified in Neurospora by measuring the phase of entrainment (PoE).
We measured period in constant darkness and PoE in 12 h light/12 h dark cycles for 98 N. crassa strains collected from between 0˚ and 47˚ latitude. The sam-ples were thus subjected to a large range of day lengths in their natural environment. We used long race tubes with a novel media formulation to ensure visualization of the circadian phenotype in wild type strains. For the Neurospora isolates, there is no significant correlation of phase with free running period in constant darkness, con-sistent with other recent results but overturning decades of basic research in mam-mals. Also, there is not a significant correlation between chronotype and latitude. The chronotype distribution of N. crassa is similar to that for our database of 30,000 Europeans showing a close to normal distribution, with few individuals (colloquially known as larks and owls) at the two extreme ends. These studies establish this sim-ple, fungal, genetic model system as a substrate for investigation of the clock as a complex trait.

591. Visualization of mitotic chromosomes and fluorescence *in situ* hybridization in *Phytophthora infestans*. Mami Kaneko and Masatoki Taga. Department of Biology, Faculty of Science, Okayama University, Okayama, Japan.

Although mitotic chromosome observation is the most basic measure of genome analysis in many eukaryotes, it has not been exploited for *P. infestans*. We developed a method to visualize mitotic chromosomes of *P. infestans* with the germ tubes from encysted zoospores. A cyst population produced by vigorously shaking zoospores was incubated to germinate on a glass slide for 2 h at 21C in 1% glucose, then the medium replaced with PDB containing both 0.02% colchicine and 0.07% colcemid to resume incubation for further 18-24 h, and finally treated with 1 mM cycloheximide for 1 h. The slide was immersed in the methanol:acetic acid (78:22) fixative, flame-dried, and fluorescence-stained with DAPI. Chromosome specimens thus prepared were mostly spread outside the burst germ tubes, making clear visualization of individual chromosomes possible under a microscope. Chromosomes were composed of the distinct, coiling two sister chromatids, and significantly long and slender. Chromosome counting with some Japanese and European strains suggested that chromosome number may not exceed 18 for each strain, but further work is necessary to draw an unambiguous conclusion. Fluorescence *in situ* hybridization with a part of 28S rRNA gene as a probe revealed the distal position of rDNA on the two chromosomes in a nucleus of a certain European strain, indicating that ploidy level of the strain is diploid.

592. The fungal gibberellin biosynthetic pathway : gene clusters and evolution. Christiane Bömke, Stefan Malonek and Bettina Tudzynski. Westfälische-Wilhelms-Universität Münster, Institut für Botanik, Schlossarten 3, D-48149 Münster, Germany

Gibberella fujikuroi is a species complex of 9 sexually fertile mating populations (MP-A to MP-I) and at least 36 anamorphic *Fusarium* species. One of these species, *Fusarium fujikuroi* (MP-C), is famous for its ability to produce high amounts of gibberellins (GAs). In the past years we identified and characterized all seven GA biosynthetic genes organized in a gene cluster. The seven enzymes catalyze 13 biosynthetic steps due to the multifunctional character especially of the four cytochrome P450 monooxygenases. Comparison of GA biosynthesis in plants and *F. fujikuroi* disproved the hypothesis of horizontal gene transfer between higher plants and fungi and confirms the suggestion that both organisms have evolved the GA biosynthetic pathways independently during evolution. We analyzed 50 species of the species complex and found out that almost all of them contain the entire GA biosynthetic gene cluster (90-98% sequence similarity), but do not produce any GAs. Interestingly, none of the so far analyzed *Fusarium* species outside the *G. fujikuroi* species complex, e.g. *F. graminearum*, contain any GA biosynthetic genes. On the other hand, fungi not closely related to the genus *Fusarium*, such as *Sphaceloma* and *Phaeosphaeria*, produce GAs. Therefore, it arises the question of the evolutionary origin of fungal GA biosynthetic gene clusters. We identified parts of a putative GA gene cluster in the genomes of fungal species not known as GA producers, such as *M. grisea* and *B. cinerea*.

593. A Genetic Selection for Circadian Output Pathway Mutations in *Neurospora crassa*. Michael W. Vitalini, Louis W. Morgan, Irene J. March and Deborah Bell-Pedersen. Center for Biological Clocks Research and Program for the Biology of Filamentous Fungi, Department of Biology, Texas A&M University, College Station, Texas 77843

To identify components of the circadian clock in Neurospora crassa, we have carried out a genetic selection to isolate mutations that alter the expression of *clock-controlled gene-1* (*ccg-1*). The promoter of *ccg-1* was fused to the *mtr* gene to create plasmid pCCG1M. The *mtr* gene encodes a neutral amino acid permease that allows for both positive and negative selection. Loss of MTR function can be selected for based on resistance to the amino acid analog p-flourophenylalanine (FPA). Gain of MTR function can be selected for based on growth of tryptophan auxotrophs on high arginine/low tryptophan (TA) media. The pCCG1M plasmid was transformed into a *bd;frq*^{10}*;mtr;trp-2* strain. Levels of *ccg-1* mRNA fluctuate randomly but remain elevated in a *frq*-null (*frq*^{10}) strain as a result of the loss of a functional FRQ-based circadian oscillator. The pCCG1M transformed strain, CCG1M, displayed the predicted Mtr+ phenotype: growth on TA media, but not on FPA. Eighty mutant strains were isolated from selective media (FPA) following UV mutagenesis of CCG1M. Analysis of some of these mutations indicates that they reside in an output pathway downstream of the FRQ oscillator. The oscillator components FRQ and WC-1 accumulate with a normal periodicity in the mutant strains; whereas genes known to require a functional FRQ oscillator for rhythmic expression (*ccg-1* and *ccg-2*) are arrhythmic in the mutant background.

594. Changes and Resources at the Fungal Genetics Stock Center. Kevin McCluskey, Sheera Walker and Mike Plamann. Fungal Genetics Stock Center, School of Biological Sciences, University of Missouri, Kansas City, Kansas City, MO.

Since the last Fungal Genetics conference, the FGSC has moved from the University of Kansas Medical Center to a newly remodeled laboratory at the University of Missouri, Kansas City. The FGSC move was largely uneventful and we have offered nearly uninterrupted service to our community. The new lab includes space for new resources housed at the FGSC including the many gene libraries from different genome projects. Among these are Neurospora, Aspergillus, Fusarium, Magnaporthe cosmid, fosmid and/or BAC libraries that are end-sequenced and mapped onto their respective genomes. Additional libraries are expected for genomes being sequenced at the Broad Institute. The FGSC has also added numerous cDNA and genomic library pools from different fungi.
With the funding of the Neurospora program project, the FGSC anticipates the addition of all the knock-out mutants as they are generated. Other systematic knock-out proposals have included the FGSC in the planning and proposal stages. As ever, the FGSC depends on the support of the fungal genetics community. If there is something that we could do, please do not hesitate to ask.

595. Isolating yeast mutants that affect mtDNA maintenance: a tool for teaching genetics and molecular biology to undergraduates. Stephan G. Zweifel. Department of Biology, Carleton College, Northfield, MN.

One of the challenges of designing an undergraduate laboratory course is to instill an appreciation of research science, yet still be able to pitch the course to students with limited laboratory experience. With its well-defined genetics and molecular biology, Saccharomyces cerevisiae is an ideal workbench for a student's first advanced laboratory course. In the sophomore level Genetics course at Carleton College, students are given the task of identifying genes responsible for the maintenance of mitochondrial DNA (mtDNA). After an introduction to basic microbial techniques, the project begins with EMS mutagenesis and a screen for temperature sensitive mutants. The experiment is a replica-plating screen based on a yeast cell's ability to grow on a non-fermentable carbon source only if functional mtDNA is present. The concepts of fermentation and respiration also provide an excellent opportunity to introduce biochemical pathways. Potential mutants are further characterized through classical genetic methods, asking such questions as: dominant or recessive, allelic to known mtDNA maintenance genes, located in the nucleus or the mitochondria. In the second half of the semester, students employ a series of cytological and molecular techniques to confirm mtDNA loss. DAPI staining and fluorescence microscopy, Southern blotting, and PCR analysis are used for the physical detection of mtDNA. The course also lends itself to an introduction to Bioinformatics with the investigation of known genes involved in mtDNA maintenance.

596. Surprising behaviour of G1 and mitotic cyclins in *Ashbya gossypii*. Katrin A. Hungerbuehler, Peter Philippsen and Amy S. Gladfelter. Biozentrum, University of Basel, Klingelbergstr. 50, CH-4056 Basel, Switzerland

The cell cycle process has been conserved throughout eukaryotes and requires temporally regulated expression, localization and degradation of cyclins and regulatory proteins. We are studying this cell cycle machinery in *A. gossypii*, a filamentous ascomycete, which is multinucleated and displays asynchronous mitoses such that neighboring nuclei are in different cell cycle stages.
The five *A.gossypii* cyclin genes have been deleted. Three cyclin genes are essential but the mutant strains were able to go through several rounds of nuclear division until they died. Localization studies showed that G1 and mitotic cyclins are nuclear but present during all cell cycle stages. However this localization alone cannot explain asynchrony of nuclear division.
One way nuclei could behave independently is if key cell cycle factors are trapped within nuclei. If nuclear sequestration is the basis for asynchrony, then how do translated proteins find the nucleus from which they were transcribed? We are investigating whether nuclear cell cycle proteins reenter their transcriptional mother nucleus. For mitotic cyclins, we showed that nuclei remain independent although proteins transcribed from one nucleus can diffuse to and enter neighboring nuclei.
Given that cyclins are freely diffusing and appear to be relatively constant in abundance, we asked whether cyclins are degraded in *A.gossypii*. A set of cyclin destruction box mutants was built, missing sequences important for degradation of cyclins in other systems. These mutants were viable and showed no growth defect unlike in other cells where stabilized cyclins led to an arrest in mitosis. These data support the existence of a novel, nuclear autonomous division cycle in which cyclin levels do not cycle.

597. The Formin Homology Protein *Ag*Bni1 is involved in tip-splitting in the filamentous fungus *Ashbya gossypii*. Hans-Peter Schmitz, Andreas Kaufmann, Michael Koehli, Philippe Laissue, and Peter Philippsen. Biozentrum, University of Basel, Klingelbergstr. 50, 4056 Basel, Switzerland

From yeast to human, Formin Homology Proteins are known nucleators of actin filaments. In order to nucleate actin cables, formins are commonly activated by Rho-type GTPases, small proteins that can act as molecular switches.
We have shown by deleting the *Ashbya gossypii* formin *AgBNI1* that this gene is crucial for hyphal morphogenesis and formation of an intact actin cytoskeleton. Germinating spores deleted for *AgBNI1* form potato-shaped cells which lack actin cables. In contrast, germlings expressing an activated form of *Ag*Bni1, in which the auto-inhibitory domain is deleted, develop into young mycelia with extensive tip-splitting prior to emergence of lateral branches – a behavior not observed in *wildtype* mycelium. Four out of the seven Rho GTPases found in the *Ashbya gossypii* genome interacted with *Ag*Bni1 in a two-hybrid assay and are thus potential regulators of the latter. Mutant alleles coding for constitutively activated Rho GTPases were introduced into the genome. Presently, we test which of these alleles may cause a tip-splitting phenotype similar to the one observed with an activated allele of *AgBNI1*.

598. Visualization of organelles in the filamentous ascomycete *Ashbya gossypii*. Dominic Hoepfner, Tineke van den Hoorn, Claudia Birrer, Ivan Schlatter, Philippe Laissue, Hans-Peter Schmitz, Philipp Knechtle, and Peter Philippsen. Biozentrum University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Schwitzerland

Organelle inheritance and dynamics are not well understood in filamentous fungi. While in single cell organisms most organelles are passed on to daughter cells by mechanisms including division prior to or during mitosis, filamentous fungi do not produce daughter cells and thus may not require elaborate organelle inheritance mechanisms. Hyphal tips of filamentous fungi continuously elongate thereby enlarging the cytoplasmic volume, and most organelles may only elongate with the growing tip. We use the model fungus *A. gossypii* to test whether organelles divide or elongate during hyphal growth and to visualize their dynamics. Previous studies on nuclear dynamics using a histone-GFP fusion revealed division kinetics comparable to *S. cerevisiae* and extensive oscillations at all nuclear cycle stages (Alberti-Segui et al. J. Cell Science 114, 975). We constructed and analyzed amino- or carboxy-terminal GFP or CFP fusions to *A. gossypii* homologs of the following *S. cerevisiae* organelle-specific proteins: Sec63 for ER, Cox4 for mitochondria, Tfp1 for vacuoles, Sec4 for secretory vesicles, Abp140 for actin cables, Cap1 and Cap2 for actin patches, Tub1 for microtubules, and CFP fused to the peroxisomal targeting sequence PTS1. We are now in the process of combining all fluorescence data in a 3D model of organelles and cytoskeletal structures in growing *A. gossypii* hyphae.

599. Septins and Mitotic Regulators in the Filamentous Ascomycete *Ashbya gossypii.* Hanspeter Helfer and Amy S. Gladfelter. Biozentrum, University of Basel, Klingelbergstr. 50, CH-4056 Basel, Switzerland

Septins are evolutionary conserved proteins with essential functions in cytokinesis, and more subtle roles throughout the cell cycle. Much of our knowledge about septins originates from studies with *S.cerevisiae,* where they form a ring-like protein scaffold at the mother-bud neck. For example, the cell cycle regulators Hsl1 and Hsl7 are recruited to this septin ring and subsequently inactivate the mitotic inhibitor Swe1, a protein kinase acting on the cyclin dependent kinase (CDK), Cdc28. The genome of the filamentous ascomycete *A.gossypii* encodes homologues of the five *S.cerevisiae* mitotic septins and all cell cycle regulators including Hsl1, Hsl7, Swe1 and the phosphatase Mih1 which counteracts the protein kinase Swe1. Nuclei in *A.gossypii* hyphae divide asynchronously and we wanted to know whether this mode of division is spatially controlled by septins, acting as cortical markers to locally direct mitosis through regulation of Swe1. We investigated the phenotypes of septin mutants with respect to septin ring formation, nuclear density, potential induction of mitosis, branching and sporulation. Deletion of single septin genes leads to loss of septin rings and sporulation but none of the deletions tested had a major impact on nuclear division frequency. To determine whether the activity of AgCdc28 is spatially controlled by septins and interacting proteins, we have started experiments to follow AgCdc28 phosphorylation in both wild type and mutant strains. Ideally these experiments will provide insights into how conserved cell cycle regulators have evolved to function in cells with diverse shapes and number of nuclei.

600. Spitzenkörper and Polarisome of *Ashbya gossypii.* Michael Köhli (1), Philipp Knechtle (1), Kamila Boudier (1), Robert Roberson (2), and Peter Philippsen (1). Biozentrum University of Basel, Klingelbergstr. 50, 4056 Basel, Switzerland (1), School of Life Sciences, Arizona State University, Tempe AZ 85287, USA (2).

Ashbya gossypii is a filamentous ascomycete. Despite its close evolutionary relation to the yeast *S. cerevisiae*, *A. gossypii* exclusively grows by apical extensions and branching. The hyphae have a diameter of 3-4 micrometers and growth speeds can reach 0.2 mm/h. We are interested in the organization of the apical growth zone which, based on genome comparison with *S. cerevisiae*, most likely carries a very similar set of proteins known to maintain yeast polar growth. A Spitzenkörper is lacking in growing *S. cerevisiae* buds and, to date, the cytoplasmic organization of the hyphal tip in *A. gossypii* is unclear. Using video enhanced light microscopy we observed an oval, phase-dense body that almost completely filled the tip zone. The behaviour of this structure was followed and analyzed during steady state growth and tip branching.
Several GFP labelled *A. gossypii* proteins locate to growing tips, e.g. homologs of the *S. cerevisiae* polarisome component Spa2, the membrane associated polar landmark protein Rax2, or the signalling protein Boi1. Based on the shape and the dynamics of these components investigated by video fluorescence microscopy, we conclude that the presumptive *A. gossypii* Spitzenkörper and the polarisome are different functional units of the apical growth machinery.

601. Stability of pesticide resistance in *Phytophthora infestans.* Stefan Bosmans, Alfons J.M. Debets, J. Arjan G.M. de Visser and Rolf F. Hoekstra. Laboratory of genetics, Wageningen UR

Mutations that confer resistance to a pesticide are generally believed to infer a fitness cost when the pesticide is no longer used. Earlier work on several organisms suggests that this cost can be compensated by additional mutations, thus leading to a stable frequency of resistant genotypes in a population. This project aims to provide insight into the stability of genetic resistance in populations of the economically important pathogen Phytophthora infestans. We will generate genotypes that are resistant to one of the following fungicidal substances: metalaxyl, fluazinam, mancozeb, dimethomorph and cyazofamid. The resistant genotypes will be generated with repeated sub-culturing on media containing sub-lethal doses. The possible fitness consequence of the several mutations will be estimated by comparing the resistant genotypes with their ancestral genotypes. Additionally, a hypothesis about the role of heterokaryons in the stability of fungicide resistance will be presented.

602. Phenotypic analysis of a deletion of the *Aspergillus nidulans* homologue of yeast *BRO1* shows involvement in the regulatory network that includes the CreB deubiquitination enzyme. Robin Lockington and Joan Kelly. School of Molecular and Biomedical Science, University of Adelaide, Adelaide, 5005, Australia.

A strain bearing a precise deletion of the *A. nidulans creA* gene, encoding the transcriptional repressor responsible for carbon catabolite repression, is viable, whereas a deletion of *creA* that extends into sequences downstream of *creA* is essentially lethal, indicating that sequences 3' of *creA* are involved in the synthetic lethality. We have identified this downstream gene as the *A. nidulans* homologue of the yeast *BRO1* gene, and named it *broA*. Precise deletion of *broA* results in a strain with pleiotropic defects including compact growth and pale conidiation on complete medium, impaired conidiation on minimal media, and impairment of the ability to utilize several carbon sources. When the *broA* deletion mutation was combined with a null mutation in the *creB* gene encoding a regulatory deubiquitinating enzyme, the *broA* deletion was found to suppress many of the effects of this mutation, implicating it as being involved in the CreB regulatory ubiquitination/ deubiquitination system involving the *creD, acrB, creB,* and *creC* gene products.

603. Sporulation in *Stagonospora nodorum*. Rohan Lowe, Peter Solomon, Richard Oliver. Australian Centre for Necrotrophic Fungal Pathogens, SABC, Murdoch University, W.A., Australia.

Stagonospora nodorum is a necrotrophic fungal pathogen that is the causal agent of leaf and glume blotch on wheat. Very little is currently known about the molecular mechanisms required for pathogenicity of *S. nodorum*, despite its major impact on Australian agriculture. *S. nodorum* is a polycyclic pathogen. Rain-splashed pycnidia attach and colonise plant tissue and sporulate within 2-3 weeks. Several cycles of infection are needed to build up inoculum for the damaging infection of flag leaves and heads. Sporulation is therefore a critical component of the infection cycle of *S. nodorum*; our aim is to determine the genetic and biochemical requirements for sporulation in order to better understand the process.
An in planta cDNA library has been constructed using transcripts isolated from infected wheat tissue. The cDNA library will be screened for genes that are expressed during the onset of sporulation. Also, the plant/fungal metabolome has been investigated during the infection process using GC/MS. Wheat plants were infected with *S. nodorum* and polar metabolites extracted from the infected tissue. Changes in metabolite levels were monitored during the onset of sporulation. Key fungal metabolites identified include mannitol and trehalose. The concentration of both mannitol and trehalose increased dramatically late in the infection period. Overall sugar levels reduced as the infection progressed, while some amino acids were observed to increase in concentration. After considering both the in planta cDNA library and metabolite analysis, several candidate genes have been selected for mutagenesis in *S. nodorum*.

604. Involvement of type 2B Ser/Thr phosphatase (Calcineurin) and PKA in sclerotial development of *Sclerotinia sclerotiorum*. Arye Harel, Rena Gorovits, Sally Bercovich, Oded Yarden. Department of Plant Pathology and Microbiology and The Otto Warburg Minerva Center for Agricultural Biotechnology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel

Sclerotia of *Sclerotinia sclerotiorum* are distinct pigmented, multihyphal structures which play a central role in the life and infection cycles of this plant pathogen. Type 2B Serine/Threonine phosphoprotein phosphatases (PP2B/calcineurin) have been demonstrated to regulate fungal cation homeostasis, morphogenesis, and pathogenesis.
To test the involvement of PP2B in sclerotial development we utilized a pharmacological and genetic approach. In the presence of the PP2B inhibitors Cyclosporin A (240nm) or FK506 (90nm), sclerotia formation was inhibited by 40 and 60%, respectively. *S. sclerotiorum* transformants harboring an inducible antisense PP2B expression construct exhibited near-normal hyphal elongation rates yet sclerotial formation was impaired.
As PP2B and cAMP-dependent protein kinase A (PKA) have been shown to be involved in at least one common pathway, we monitored changes in relative PKA activity levels during sclerotial development. Relative PKA activity levels increased during the white-sclerotium stage in the wild-type strain, while low levels were maintained in non-sclerotium-producing mutants. The changes in PKA activity that accompany sclerotial development in a distinct developmental phase manner and the requirement of PP2B for sclerotial development, indicate the involvement of these components, in addition to other signal transduction pathways, in the regulation of sclerotiogenesis.

605. On the evolution and ecology of fungal senescence. Marc F. P. M. Maas, Rolf F. Hoekstra and and Alfons J.M. Debets. Laboratory of Genetics, Wageningen University, The Netherlands

Filamentous fungi, being typical modular organisms, are not expected to age and die. Nonetheless there are some striking examples of aging or senescence in these fungi. Most, if not all of the cases thus far described are associated with instability of the mitochondrial genome and recent evidence has shown that this perhaps results from mitochondrial oxidative metabolism. Calorie restriction (CR) is the only life span extending regimen known that applies to all aging organisms, and also extends life span in P. anserina. Natural variation in the life span extending response of CR can in the latter species largely be explained by the presence of pAL2-1 homologous mitochondrial invertrons. The pAL2-1 homologues drastically reduce life span and are effectively similar to the structurally related plasmids pKALILO or pMARANHAR from Neurospora. Probably their effect is in the shadow of other aging factors and is drawn out of that shadow by CR, explaining why it has thus far been overlooked. We argue that fungal senescence is from an ultimate perspective, aging in the true sense of the word.

606. Agrobacterium Tumefaciens – Mediated Genetic Transformation of the Edible Straw Mushroom Volvariella volvacea.
Tran Hoang Ngoc Ai, Hoang Quoc Khanh*. Institude of Tropical Biology, Vietnam Academy of sciences and Technology

Agrobacterium tumefaciens is known to transfer parts of its tumor – inducing plasmid , the T- DNA, to plants, yeasts and filamentous fungi; therefore, we have used this system to transform germinating basidiospores and vegetative mycelium of the cultivated basidiomycete *Volvariella volvacea.*

Plasmid pPK2 contains hygromycin hph gene (B phosphotransferase) with the *Aspergillus nidulans* gpd promoter (glyceraldehyd - 3 - phosphate dehydrogenase). Hph gene which is 1 kb long was designed between LB and RB of T-DNA in this plasmid DNA. Out of T-DNA is kanamycin resistant gene, used to select transformed bacteria.

Spores of *V. volvacea* were collected on glass Petri dishes. The standard growth medium for *V. volvacea* was PDA and incubation was performed at 28oC.

pPK2 was transformed into *A. tumefaciens* via electroporation. Bacterial cultivation was performed as describe in De Groot et al. (1998). For induction of virulence and T-DNA transfer, *A. tumefaciens* was grown on induction medium (IM) with 200 mM AS and, for negative controls, without AS. Selection for transformed mycelial colonies was performed on M-100 agar containing cefotaxime (500 mg/ml) and hygromycin B (70 mg/ml) and on CYM –R containing hygromycinB (70m/ml). Hph gene sequence of T-DNA which integrated genomic DNA of *V. volvacea* was checked by PCR. Analysis of transformants shows that the T-DNA integrates at random sites into the host genome and that the selection marker is stable during mitosis and meiosis.

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