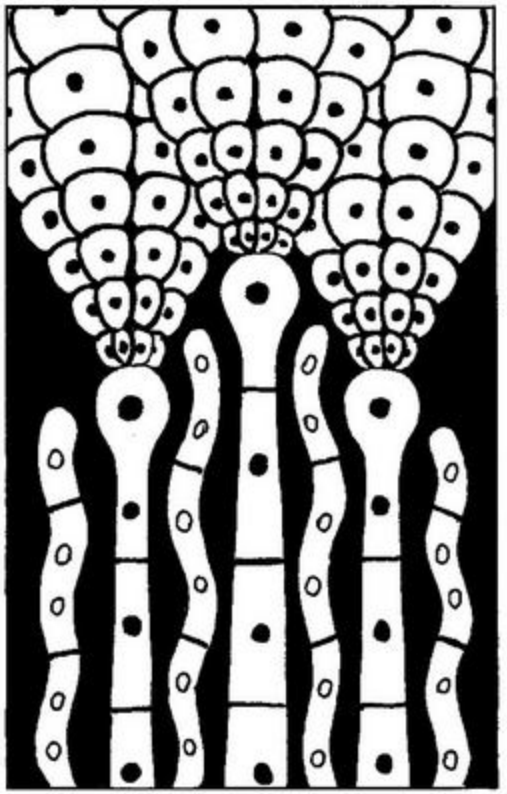
Announcement

Scientific Program

Plenary Abstracts
Poster Abstracts

Activities at Asilomar

FUNGAL



GENETICS

ASILOMAR MARCH 2007

24th Fungal Genetics Conference

March 20-25, 2007
Asilomar Conference Center, Pacific Grove, CA

CHAIRS OF THE SCIENTIFIC PROGRAM

Dr. Barbara Howlett
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Aspergillus Satellite Meeting

Workshops

Handouts from the teaching workshop

The Fungal Genetics Policy Committee invites you to attend the 24th Fungal Genetics Conference, sponsored by the Genetics Society of America.

The meeting will be at the Asilomar Conference Center, Pacific Grove, California (near Monterey, California).

The conference will open on Tuesday evening, March 20 with an Opening Mixer from 7:30 pm – 10:30 pm and end on Sunday, March 25 at 12:00 pm.

All meeting questions should be directed to Anne Marie Mahoney, GSA Meetings Manager, annemarie.Mahoney@verizon.net, Genetics Society of America, 9650 Rockville Pike, Bethesda, Maryland 20814.

Chairs of the Scientific Program:

Dr. Barbara Howlett	Dr. Joseph Heitman
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Conference Deadlines:

Financial Aid Application	November 15, 2006
Perkins Fund Award Application	November 29, 2006
Abstract Submission	December 13, 2006
Meeting and Housing Registration	December 13, 2006

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ABSTRACT SUBMISSIONS

Abstract submissions will be online at the FGSC web site beginning on October 27, 2006. Complete instructions for electronic submission of poster abstracts will be available when the site opens. If you encounter difficulties in submitting your abstract via the web, contact: Kevin McCluskey, PhD, Curator FGSC, at http://www.fgsc.net/contact.cfm.

PLENARY SESSIONS

The chairs have organized the plenary sessions. Each session will consist of five invited talks of general interest.

CONCURRENT SESSIONS

There will be four sets of concurrent sessions on a variety of topics. Twenty-five sessions are planned and are listed in the tentative conference schedule.

POSTER SESSIONS

Deadline for receipt of abstracts: December 13, 2006. All abstracts must be submitted via the web. Poster Sessions will be in the evenings, March 21-23. The size of the poster should not exceed 4 feet (112 cm) x 4 feet (112 cm). Two authors will share a 4-foot high x 8-foot wide poster board. No materials for making posters will be available at the conference grounds. We encourage photos of the authors be attached to posters to facilitate identification by new members of the group.

If you submit an abstract, you will be expected to bring a poster. Concurrent session organizers will have early access to the abstracts and may invite additional speakers from the abstract pool to speak. You are still expected to bring a poster if you are asked to give a talk. If you are an invited concurrent session speaker, you do not need to submit an abstract unless you are also bringing a poster.

AD HOC SESSIONS

Time is available in the early afternoons and Sunday morning for ad hoc meetings. Those wishing to convene such informal sessions should contact one of the scientific chairs.

AUDIO VISUAL

The following equipment will be provided in all sessions: an LCD projector, laser pointer and lavaliere microphone. Presenters should plan to be in Merrill Hall 60 minutes prior to the start of their plenary session to load their presentation.

POSTER SESSION AWARDS

Awards will be given for the best poster presentations. Prizes will be given to the students or postdocs judged to have the best posters. Both scientific merit and clarity of presentation will be taken into account. The awards will be presented at the Closing Banquet on Saturday, March 24.

VISAS

The National Academies has a useful web site concerning obtaining a visa (http://national-academies.org/visas). As part of new security procedures, many applications are being sent to the State Department in Washington where they are reviewed, with assistance from other agencies. Because of the number of visas being processed and the need to be thorough with the reviews, this can take as much as 3 to 4 months. Therefore, we advise scientists intending to come to the United States to apply for their visa as early as possible.

FINANCIAL SUPPORT

Some funds will be available to support the participation of students and others that might otherwise be unable to attend. Awards are generally limited to room and board, although in special circumstances, travel assistance may also be available. If you wish to be considered for such support, please submit the Financial Support Application (doc or pdf) form. All applicants will be notified of financial award decisions in time to register for the meeting. Deadline for receipt of financial assistance requests is November 15, 2006.

TRAVEL

Monterey is the closest commercial airport to Asilomar. Taxis meet all arriving flights and cost approximately $25 per person. The airport is about 20 minutes from Asilomar. The closest major airports are, in order of convenience, San Jose, San Francisco and Oakland.

For maps and directions to Asilomar please go to:
http://www.visitasilomar.com/maps.aspx

PERKINS FUND AWARD

The Perkins Fund Award allows graduate students or postdoctoral scientists that utilize Neurospora as an experimental organism to attend the Fungal Genetics Conference or the Neurospora Conference. It is now time to solicit the fifth round of nominations for Perkins Fund Awardees.

As a result of your generosity, the fund can support two awards to attend the upcoming 24th Fungal Genetics Conference. Awards of approximately $400 each will go to one graduate student and one postdoctoral scientist. Nominations for Perkins Fund Awardees should be made in the form of a one-page letter from the candidate's research advisor. This letter should include a 200 word description of why the

individual's work is exceptional. Up to two re-prints or pre-prints of published research may also be included with the nomination.

Evaluation of the nominations will be overseen by the Neurospora Policy Committee. The awardees will be notified prior to the meeting, and the award will be presented at the banquet on the final night of the meeting.

The nomination letter and any accompanying materials should be sent as a PDF via e-mail no later than November 29, 2006 and should be sent to: Dr. Oded Yarden, Chair Neurospora Policy Committee, E-mail: Oded.Yarden@huji.ac.il

REGISTRATION

Deadline for conference registration and housing reservations is December 13, 2006. Neither submission of an abstract nor GSA membership is required for attendance at the meeting. If you wish to join GSA, you may do so online at the GSA web site: http://www.genetics-gsa.org (click on GSA Membership/Joining the GSA in the menu on the left of your screen).

Conference registration will be accepted via the Fungal Conference Online Registration website opening on October 27, 2006 or by mailing in the appropriate form (doc or pdf). Payment may be made by Visa, MasterCard, American Express or Discover.

Registration Fees:

GSA member/affiliate	$185
Nonmember	$325
Post docs	$155
Post doc nonmember	$255
Student	$130
Student nonmember	$190
Accompanying Guest	$0

If you choose to mail in a registration form, it will be processed ONLY if accompanied by payment, which may be made by a credit card or a check drawn on a US bank, payable to the Genetics Society of America. Purchase orders will not be accepted. Credit card payments will be accepted by fax, but not by telephone.

A $25 processing fee will be charged for all meeting registration cancellations. There will be no refunds for cancellations received after February 11, 2007. Cancellations must be in writing, an e-mail is sufficient. Cancellation notices should be sent to annemarie.mahoney@verizon.net. There will be a $50 fee for late registrations and these applications will be considered on a space available basis.

Roommate Bulletin Board

An electronic bulletin board is available on the online registration webpage to facilitate finding a roommate. Registrants can post messages regarding what type of roommate they are looking for (male or female to share a double, triple or quad) and the participants will respond directly to the registrant.

Oversubscription Policy

Space is limited at Asilomar Conference Center to 750 participants. To encourage participation by as many labs as possible, IN THE EVENT THAT THE MEETING IS OVERSUBSCRIBED, there will be a limit of four registrants from any one lab. For labs with multiple registration submissions, priority will be given to more senior graduate students and postdoctorals. If limiting attendance is necessary, first year graduate student registrations will be considered lowest priority, as there will be opportunities for them to attend a future meeting.

HOUSING

Asilomar Conference Center is located on 107 acres of forest, dune and beach at the tip of the Monterey Peninsula. Asilomar retains many of the original flora and fauna of the peninsula. Please go to www.visitAsilomar.com for detailed information about the area. The weather is brisk (40-60◦ F) with a possibility of rain. Bring warm clothing and a raincoat.

Accommodations

All rooms have private baths. There are very few singles available. Requests for single rooms will be honored in the order received. All rates include meals, beginning with Tuesday dinner and concluding with Sunday lunch. No refunds are available for missed meals. Infants under age 2 years of age may stay in your room with no charge. Children from 3-17 years old are charged the special rate of $306 provided there is triple or quadruple occupancy with one adult in certain rooms. Otherwise, they pay regular rates. No pets are allowed on the grounds.

Housing Preference	Fee	Housing Preference	Fee
Single	$865	Quadruple	$410
Double	$545	Children	$306
Triple	$420		

In addition to the rooms at Asilomar a limited number of people will be housed at the Deer Haven Inn. The Deer Haven Inn is located across the street from Asilomar. The cost will be the same as staying on the Asilomar grounds. For those assigned to the hotel, an Asilomar meal card will be given to the attendee when they check in at the Conference Registration Desk.

Every attempt will be made to honor your housing request. Due to Asilomar room limitations it may be necessary to move you to a different rooming category. You will be charged accordingly.

If an attendee would like to bring a guest, the guest MUST fill in a registration/housing reservation form and indicate their roommate on the form. Please be sure that both people indicate each other as roommates. The guest does not need to pay a meeting registration fee.

Check-in

Check-in begins at 3:00 pm on Tuesday, March 20 in the Administration Building. Check-out is noon on Sunday, March 25.

Housing Cancellations

If you must cancel your housing, please advise Anne Marie Mahoney, GSA Meetings Manager, annemarie.mahoney@verizon.net immediately. Individuals who cancel after February 11, 2007 will not be entitled to a refund unless a substitution can be made.

Special Meals

There will be a Closing Banquet on Saturday, March 24. Please indicate your meal preference on the registration form. Anyone who is staying at Asilomar but not attending the conference can purchase a ticket for the banquet on Saturday night for $25. On Sunday, March 25, box lunches will be available. Please indicate on the registration if you would like a box lunch.

Telephone and Mail

There are no phones in the rooms. The address and phone number is C/o Fungal Genetics Conference, Asilomar Conference Center, PO Box 537, Pacific Grove, CA 93950, Phone (831) 372-8016, Fax: (831) 372-7227.

ADDITIONAL FEES

For individuals who choose to make their own hotel reservation, a meal package is available for $235. This rate includes dinner on Tuesday, March 20 through lunch on Sunday, March 25 and the Asilomar facilities fee.

24th Fungal Genetics Conference Scientific Program

Tuesday, March 20

3:00 pm – 10:00 pm Registration **Administration Building**
3:00 pm – 5:30 pm *Sclerotinia/Botrytis* Annotation ad hoc workshop **Triton**
6:00 Dinner, **Crocker Hall**
7:30 pm – 9:30 pm The Perkins and Stadler Memorial Celebration **Chapel**
7:30 pm – 10:30 pm Social Reception (Mixer) **Merrill Hall**

Wednesday, March 21

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**

Genome Structures and Dynamics Chair, David Catcheside

Ken Wolfe The *Kluyveromyces polysporus* genome: gene losses, convergence of genome contents,
and the origin of yeast species.
Bettina Tudzynski Evolution of gene clusters in fungi: lessons from isoprenoid gene clusters
Michael Freitag Genome defense by repeat-induced point mutation: an evolutionary dead end?
Laura Rusche Evolution of silencing mechanisms in Hemiascomycetes
Bill Holloman DNA Repair and Recombination in *Ustilago maydis*

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.

12:15 pm – 2:00 pm**, Ad hoc workshops**

Fusarium graminearum **Heather**
The *Phycomyces* genome: manual annotation and analysis **Triton**

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

Associations between fungi and humans Aaron Mitchell and Maurizio del Poeta **Merrill Hall**
Signal transduction and cell surface receptors Kathy Borkovich and Tom Fowler **Fred Farr Forum**
Population Genetics Matthew Fisher and Mary Berbee **Chapel**

Regulation of primary and secondary metabolism Joan Kelly and Ullrich Kuck **Kiln**
Industrial mycology in the post-genomics era Joan Bennett and Merja Penttila **Nautilus**
Dimorphic transitions Anne Dranginis and Alex Andrianopoulos **Heather**
Teaching fungal biology and genetics Pat Pukkila and Amy Reese **Triton**

6:00 Dinner, **Crocker Hall**

7:30 pm – 10:30 pm Poster Session I

Poster Topics
Cell Biology
Population and Evolutionary Biology
Animal and Plant Pathogens; Fungal-Host interactions

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

Thursday, March 22

7:30 am - 1:00 pm Registration, **Administration**
7:30 am - 8:30 am Breakfast, **Crocker Hall**
8:30 am – 12:00 pm Plenary Session II, **Merrill Hall**

Host-pathogen and symbiotic interactions Chair, Marc Orbach

Jim Beynon The Remarkably Diverse Effector Proteins of *Hyaloperonospora parasitica*
Barry Scott Molecular insights into mutualism in a fungal-plant interaction
Jin-Rong Xu *Magnaporthe grisea* infection of rice and parallel studies in wheat scab
Joyce Longcore *Batrachochytrium dendrobatidis*, the Chytrid Pathogen of Amphibians
Jenny Lodge Molecular dissection of the cell wall of *Cryptococcus*

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

12:00- 1:00 pm Neurospora Business 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.

12:15 pm – 2:00 pm**, Ad hoc workshops**

Nectria haematococca Sequence **Nautilus**
Dothideomycete Genome Sequence **Heather**
Planning Session on functional characterization of orphan genes and hypothetical proteins **Kiln**

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

Fungal-plant interactions Scott Gold and Jan van Kan **Merrill Hall**
Mating and sexual development JP Xu and Takashi Kamada **Nautilus**
Biological applications of genomic sequence data microarrays and expression analysis – Al Brown and Anita Sil **Chapel**
Circadian rhythms and photobiology Sue Crosthwaite and Reinhard Fischer **Fred Farr Forum**
Biofilms, quorum sensing and thigmotropism Christophe d'Enfert and Neil Gow **Kiln**
Zygomycete and chytrid genomics, biotechnology and evolutionary biology Scott Baker and Tim James **Heather**

6:00 Dinner, **Crocker Hall**

7:30 pm – 10:30 pm Poster Session II

Poster Topics
Genomics and Proteomics
Biochemistry and secondary metabolism
Developmental Biology
Model systems
Authors of ODD numbered posters should be at their poster from 7:30- 8:30 and authors of EVEN numbered posters should be at their posters from 8:30 - 9:30.

Friday, March 23

7:30 am - 1:00 pm Registration, **Administration**
7:30 am - 8:30 am Breakfast, **Crocker Hall**
8:30 am – 12:00 pm Plenary Session III, **Merrill Hall**

Development and metabolism Chair, Axel Brakhage

Michael Hynes Transcriptional controls of carbon source utilization in *Aspergillus nidulans*
Jae Hyuk Yu Growth and developmental control in *A. nidulans* and *A. fumigatus*
Marty Dickman The necrotrophic fungus, *Sclerotinia sclerotiorum* subverts host pathways by inducing apoptosis for disease development.
Luis Corrochano An intelligent primitive eukaryote: environmental sensing in *Phycomyces*
Deborah Hogan Quorum sensing in bacterial-fungal interactions

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.

12:15 pm – 2:00 pm, **Ad hoc workshops**

Making gene ontology annotations for fungal genomes **Nautilus**
Heterobasidion genome sequence **Kiln**

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

Epigenetics and genome dynamics (& transposons) Emmanuelle Fabre and Ed Louis **Fred Farr Forum**
Whole genome comparative analysis Jim Kronstad and Cecile Fairhead **Merrill Hall**
Proteome and post-genomic approaches to protein secretion Dan Cullen and Debbie Fox **Kiln**
Small molecules and signaling Gillian Turgeon and Hubertus Haas **Chapel**
Apoptosis and vegetative incompatibility Louise Glass and Marty Dickman **Nautilus**
Advances in oomycete research Francine Govers and Brett Tyler **Heather**

6:00 Dinner, **Crocker Hall**

7:30 pm – 10:30 pm Poster Session III

Poster Topics
Industrial Biology and Biotechnology
Gene Regulation
Animal and Plant Pathogens; Fungal-Host interactions
Others

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

Saturday, March 24

7:30 am - 1:00 pm Registration, **Administration**
7:30 am - 8:30 am Breakfast, **Crocker Hall**
8:30 am – 12:00 pm Plenary Session IV, **Merrill Hall**

Sex, time and evolution Chair, Linda Kohn

Leah Cowen Protein Folding, Environmental Contingency, and Evolution: Hsp90's Role in Fungal Drug Resistance
John Taylor Comparative genomics of *Coccidioides* species
Deborah Bell-Pederson Unwinding the *Neurospora* circadian clock
Christina Hull Sex: how *Cryptococcus neoformans* controls itself
Arturo Casadevall Origin and maintenance of virulence in human pathogenic fungi

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

Cool tools for fungal biology: gene disruption and imaging Matt Sachs and Nick Read **Chapel**
Evolutionary genetics and genomics Jim Anderson and Dee Carter **Merrill Hall**
Symbiotic and parasitic (viruses, nematodes, other fungi and insects) interactions Teresa Pawlowska and Don Nuss **Nautilus**
RNA functions Michael Feldbrugge and Marcus Kuenzler **Kiln**
Cell morphogenesis and development Amy Gladfelter and Michelle Momany **Fred Farr Forum**
Basidiomycete biology Regine Kahmann and Lorna Casselton **Heather**

6:00 Banquet, **Crocker Hall**

8:00 pm – 9:00 pm Invited Lecture June Kwon Chung, US NIH

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 25

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

8:30 am **Ad hoc Workshops**
Comparative genomics of the fusaria and organization of a *Fusarium* working group **Kiln**
Colletotrichum Workshop **Sanderling**

12:00 pm Check-out

XXIV Fungal Genetics Conference at Asilomar

Plenary Session Abstracts

Genome Structures and Dynamics

The *Kluyveromyces polysporus* genome: gene losses, convergence of genome contents, and the origin of yeast species.

Kenneth H. Wolfe. Smurfit Institute of Genetics, Trinity College, Dublin 2, Ireland. khwolfe@tcd.ie

We report an 8x draft genome sequence of *Kluyveromyces polysporus*, a yeast species that underwent whole-genome duplication (WGD) but represents the clade of post-WGD species that is most distantly related to *Saccharomyces cerevisiae*. We find that the subsequent process of loss of superfluous copies of genes in *K. polysporus* proceeded almost independently of that in *S. cerevisiae*. At the time the two lineages diverged, their common ancestor still retained about 9000 genes and 80% of them were members of duplicated pairs resulting from the WGD. The genomes of the two lineages then shrank independently and converged onto their current size of around 5600 genes each. The functional categories of genes that survived in duplicate in the two genomes are similar, confirming the role of natural selection in this process, but at loci where each species retained only one copy they show almost random choice of which copy to retain. Thus, *K. polysporus* contains pairs of duplicated chromosomal regions that are superficially similar to those in *S. cerevisiae*, but whose actual makeup is very different. About 45% of the single-copy genes in these two species are paralogs, not orthologs. These observations support the hypothesis that two polyploid lineages can become reproductively isolated from one another if they lose alternative copies of duplicated genes (Dobzhansky-Muller incompatibility).

Evolution of gene clusters in fungi: lessons from isoprenoid gene clusters.

Christiane Bömke and Bettina Tudzynski, Universität Münster, Institut für Botanik, Schlossgarten 3, D-48149 Münster, Germany

With the availability of fungal genome sequences, we are now able to gain insights into the evolution of gene clusters for biosynthesis of secondary metabolites. Horizontal as well as vertical transmission of gene clusters, followed by selective loss during evolution have been proposed to explain the existence of similar clusters in distantly, and theír missing in closely related species.

We work on identification of gibberellin (GA)- and other isoprenoid gene clusters inside and outside the genus *Fusarium*. After disproving the hypothesis of horizontal gene transfer between higher plants and fungi, we suggest that both groups of organisms have evolved the GA biosynthesis pathways independently. Analyzing about 50 *Fusarium* species, we showed that only the closely related members of the *Gibberella fujikuroi* species complex, but not other *Fusarium* species, contain the GA gene cluster. On the other hand, fungi not closely related to the genus *Fusarium*, such as *Sphaceloma* and *Phaeosphaeria,* produce GAs. *Magnaporthe grisea* contains an active GA-specific diterpene cyclase in a rudimentary cluster. In *Phoma betae,* a similar gene cluster have been identified which is responsible for the production of the GA-like compound aphidicolin. The conserved intron positions in the diterpene cyclase genes and the physical linkage to a pathway-specific GGPP synthase gene let us assume that fungal diterpenoid gene clusters have one phylogenetic origin.

Genome defense by repeat-induced point mutation: an evolutionary dead end?

Michael Freitag. Dept. of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, USA

In 1987, Eric Selker and colleagues uncovered the first eukaryotic “genome defense” system, a hypermutation phenomenon later dubbed “repeat-induced point mutation” (RIP). This premeiotic process detects and inactivates

duplicated DNA segments of varying length, i.e. gene-sized to large chromosome segments. RIP takes place after fertilization, during a stage characterized by pre-meiotic nuclear divisions, but before karyogamy occurs. The RIP machinery peppers both copies of duplicated sequences with C:G to T:A transition mutations. While the mechanisms involved in homologous DNA pairing and the subsequent mutagenesis remain obscure, RIP has recently enjoyed much attention because of its potential role in shaping fungal genomes. RIP appears evolutionary conserved within the true ascomycetes because at least one essential component of the RIP machinery, the putative DNA methyltransferase RID, is retained in many taxa. Moreover, active RIP has by now been demonstrated in several ascomycetes and putative relics of RIP have been found in many additional species. Evolutionary implications and our work with mutants defective in RIP will be discussed.

Evolution of Silencing Proteins in Hemiascomycetes.

Laura Rusche and Meleah Hickman, Duke University, Durham, North Carolina

In *S. cerevisiae*, the mating type of haploid yeast is determined by the *MAT* locus, which can express **a** or alpha genes, encoding master transcriptional regulators. To enable the cells to switch mating type, the **a** and alpha genes are also encoded at the silent mating-type loci, *HMR**a*** and *HMLα*. These two loci are constitutively silenced by the Sir proteins. Two of the four Sir proteins, Sir2p and Sir3p, have paralogs that arose in a whole-genome duplication that occurred in the ancestry of *Saccharomyces*. Hence, this duplication event may have enabled the Sir proteins to become more specialized for silencing. We are exploring the evolution of the Sir proteins by investigating the functions of Sir homologues in two species, *Kluyveromyces lactis* and *Ashbya gossypii*, which diverged from *S. cerevisiae* prior to the whole-genome duplication.

DNA Repair and Recombination in *Ustilago maydis*.

M. Kojic, Q. Zhou, N. Mazloum, N. Mao, and W. K. Holloman, Department of Microbiology and Immunology, Cornell University Weill Medical College, New York, NY 10021

Genome stability relies on a network of interacting systems that detect and repair disturbances in the integrity of DNA. Inactivation or impairment of these systems can lead to chromosome aberrations, mutation, and death. In many cases elements of these systems are highly conserved, indicating universality of the mechanisms and by inference the importance of these systems in preserving the genome of the hosts. A central pathway that processes potentially lethal types of DNA damage employs a mechanism that enables repair by homologous recombination. Components of this pathway in eukaryotes such as *Homo sapiens* include Rad51 whose function is to search for DNA sequence homology and promote strand exchange, plus factors that enable and enhance Rad51's activity. These latter include the product of the BRCA2 breast cancer susceptibility gene, that serves as the key regulator of Rad51, and also the Rad51 paralogs, proteins structurally related to Rad51 that serve to promote its function. In the emerging view, a DNA molecule sustaining a double-strand break is resected from the exposed duplex ends to reveal protruding single-stranded stretches. These pair with a homologous DNA sequence, after which DNA synthesis proceeds with the undamaged homologous sequence serving as a template to fill in the missing nucleotides. Following resolution of the joint molecule intermediates or dissociation and reannealing of the invading strand the repair process is completed.

We are interested in recombinational repair processes in *Ustilago maydis,* a basidiomycete phytopathogenic fungus that has served as a model organism for the elucidation of the central mechanism of recombinational repair for many years. The recombinational repair system in *U. maydis* relies on orthologs of the human Rad51 protein, BRCA2 (termed Brh2 in *U. maydis*), and Dss1. Brh2 enables recombinational repair of DNA by controlling Rad51 and is in turn regulated by Dss1, a small polypeptide. Interplay with Rad51 is conducted via the BRC element located in the N-terminal region of the protein and through an unrelated domain, CRE, at the C-terminus. Mutation in either BRC or CRE severely reduces functional activity, but repair deficiency of the *brh2* mutant can be complemented by expressing BRC and CRE on different molecules. This intermolecular complementation is dependent upon the presence of Dss1. Brh2 molecules associate through the region overlapping with the Dss1-interacting domain to form at least dimer size complexes, which in turn, can be dissociated by Dss1 to monomer. The model that emerges

from this work proposes that cooperation between BRC and CRE domains and the Dss1-provoked dissociation of Brh2 complexes are requisite features of Brh2's molecular mechanism.

Host-pathogen and symbiotic interactions

The Remarkably Diverse Effector Proteins of *Hyaloperonospora parasitica*.

Jim Beynon, Rebecca Allen, Laura Baxter, Rachel Baumber, Peter Bittner-Eddy, Mary Coates, Kate Fisher, Sharon Hall, Linda Hughes, Sarah Lee, Julia Meitz, Anne Rehmany and Laura Rose[1]. Warwick HRI, Warwick University, Wellesbourne, Warwick, CV35 9EF, UK. [1]Department of Evolutionary Biology, University of Munich, Großhadernerstr. 2, 82152 Planegg-Martinsried, Germany

Hyaloperonospora parasitica is an obligate oomycete and the causal agent of downy mildew on Arabidopsis. Recently we have cloned two pathogenicity effector genes, *ATR1* and *ATR13*, from the pathogen and shown them to code for unique proteins that are under amazing levels of diversifying selection. This implies that they are locked in an "arms race" with the plant's pathogen detection system and consequently these levels of diversity are mirrored in the host resistance genes, *RPP1* and *RPP13*, associated with recognition of ATR1 and ATR13, respecitively. We have used this natural variation to identify key amino acids involved in determining specificity in the ATR13/RPP13 interaction. Using bioinformatic analyses we have identified a further 140 candidate effectors and they reveal a fascinating picture of the effects of diversifying selection presumably resulting from their interactions with Arabidopsis defence mechanisms. It is difficult to ascribe all the diversity seen between *ATR13* alleles to the presence of a single host resistance gene and we will describe the complexity of the ATR13/RPP13 interaction.

Molecular insights into mutualism in a fungal-plant interaction.

Barry Scott, Daigo Takemoto, Aiko Tanaka, and Carla Eaton. Massey University, New Zealand.

Key requirements for microbes to initiate and establish mutualistic symbiotic interactions with plants are evasion of potential host defense responses and strict control of microbial growth. We have recently shown that reactive oxygen species (ROS) produced by a specific fungal NADPH oxidase isoform NoxA, have a critical role in regulating hyphal growth in the mutualistic interaction between *Epichloë festucae* and perennial ryegrass (Tanaka et al. 2006). In professional phagocytes, activation of the membrane associated NADPH oxidase ($gp91^{phox}$) involves cytosolic recruitment of a heterotrimeric phagocyte oxidase complex (phox) and the small GTP binding protein Rac2 in response to microbial or inflammation signals. *E. festucae* homologues of $p67^{phox}$ and *Rac2*, designated *noxR* and *racA*, were cloned and deletions of each generated (Takemoto et al. 2006; Tanaka et al. unpublished). Symbiota containing these mutants had a similar stunting phenotype to *noxA*. Hyphae in these symbiota were hyper-banched and the biomass dramatically increased. Over-expression of *noxR* results in hyper-branching of *E. festucae* in culture, as does treatment of wild-type cells with the ROS inhibitor DPI. Using yeast two-hybrid and pull-down assays NoxR was shown to interact with RacA. A single amino acid substitution in the predicted RacA-binding site of NoxR (R101E) abolished this interaction. A *noxR* construct containing this mutation failed to complement the *noxR* mutation *in planta*. Taken together these data demonstrate that NoxR is a key regulator of NoxA in the symbiosis, where it acts together with RacA to spatially regulate ROS production and control hyphal growth and patterning.

***Magnaporthe grisea* infection of rice and parallel studies in wheat scab.**

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Rice blast caused by *Magnaporthe grisea* is one of the most severe fungal diseases of rice throughout the world. In the past decade, it has been developed as a model system to study fungal-plant interactions. Several signal transduction pathways regulating infection-related morphogenesis and fungal-plant interactions have been identified. The *PMK1* MAP kinase is essential for infectious growth and the formation of the highly specialized infection structure known as appressorium. Several upstream components of the *PMK1* pathway, including *MST7, MST11, MST50, RAS2,* and *MGB1*, were identified and functionally characterized. One downstream transcription factor *MST12* and a few virulence factors regulated by *PMK1* also have been characterized. Our results indicate that well conserved signaling pathways may have different upstream signal inputs and outputs in fungal pathogens. A MAP kinase pathway homologous to *PMK1* also is essential for pathogenesis in *Fusarium graminearum*, the causal agent of wheat and barley scab. Microarray analysis was used to compare genes regulated by this MAP kinase pathway in these two pathogens with distinct infection mechanisms. A few genes commonly regulated by *PMK1* and its homolog in *F. graminearum* were selected for functional analysis. Several pathogenicity factors known in *M. grisea* also were analyzed for their roles in *F. graminearum*. One of them is the *TBL1* transducin-beta gene that is required for infectious growth after the initial invasion.

***Batrachochytrium dendrobatidis*, the Chytrid Pathogen of Amphibians.**

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Batrachochytrium dendrobatidis (*Bd*), described in 1999, is an amphibian pathogen and the probable cause of declines of amphibian populations on five continents. In addition to numerous genera and species in Australia and Central America, examples of affected species are the mountain yellow-legged frog in California, the midwife toad in Spain and the Kihansi spray toad in Africa; however, infection also can be present without seeming to affect population numbers. *Bd*, the only chytrid pathogen of vertebrates, develops determinant, spherical thalli within keratinized skin cells of hosts; at maturity each sporangium produces a discharge papilla that extends to the skin surface. The chytrid releases motile zoospores into the exterior environment. *Bd* also can live without an amphibian host. In pure culture, individual thalli with thread-like rhizoids grow up to 40 μm dia on 1 % tryptone medium, and mature in ~ 4 days at 23 C. During maturation the entire nucleated cytoplasm divides into asexual zoospores, which exit the zoosporangium through 1-several discharge papillae. Sexual reproduction is unknown, and is rarely reported in the *Rhizophydiales*, the order in which this species is classified. Field research has contributed to knowledge of the distribution and effects of *Bd* but only pure cultures have made possible fulfillment of Koch's Postulates, experimental inoculations, progress in methods to detect *Bd*, studies of population genetics and whole genome sequencing.

Molecular dissection of the cell wall of *Cryptococcus*

Lorina Baker, Charles Specht, Isaac Banks and Jennifer Lodge

The fungal cell wall provides structure and protection from the environment, and it is intimately involved with interactions with host cells during infection. Fungal walls are composed of various polysaccharides and proteins, and virulence factors are often cell wall associated. Exposure of specific wall polysaccharides on the cell surface provoke specific host responses. Chitin, a rigid, insoluble, polymer of N-acetylglucosamine, has been shown to be an essential component of the cell wall of many fungi. Chitin also can be enzymatically deacetylated to chitosan, a more flexible and soluble polymer. *Cryptococcus neoformans* is a fungal pathogen that causes cryptococcal meningoencephalitis, particularly in immunocompromised patients. We have shown that both chitin and chitosan are present in the cell wall of vegetatively growing *C. neoformans* yeast cells, and that the levels of both rise dramatically as cells grow to higher density in liquid culture. Chitosan is also present during when the fungi is growing in a mouse model. Although *C. neoformans* has eight putative chitin synthases, and none are essential, one of them, Chs3, appears to produce the bulk of the chitin which is converted to chitosan. Deletion of a putative regulator of chitin synthase also substantially reduces the production of chitosan. We have also identified three chitin deacetylases that account for all of the chitosan in the wall. The data suggests a model for chitosan production in vegetatively growing *C. neoformans* where the three chitin deacetylases convert chitin generated by the chitin synthase, Chs3, into chitosan. In *C. neoformans*, chitosan helps to maintain cell integrity and aids in bud separation. Additionally, chitosan is necessary for maintaining normal capsule width, and lack of chitosan results in a "leaky

melanin" phenotype. Mutants in the chitin deacetylases are less viable in the mouse model, suggesting that chitosan is critical for *C. neoformans* interactions with its host.

Development and metabolism

Transcriptional controls of carbon source utilization in *Aspergillus nidulans*.

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In *Aspergillus nidulans*, acetate utilisation involves acetyl-CoA formation in the cytoplasm and metabolism via the glyoxalate cycle in peroxisomes and via the TCA cycle in mitochondria. A cytoplasmic carnitine-acetyl transferase allows acetyl-CoA to enter the organelles as acetyl-carnitine which is converted back to acetyl-CoA by a second carnitine-acetyl transferase encoded by *acuJ*, a gene required for growth on both acetate and fatty acids which are converted to acetyl-CoA by peroxisomal beta-oxidation. A peroxisomal targeting mutation results in loss of growth on fatty acids indicating that AcuJ is necessary for transport of acetyl-CoA from peroxisomes to the mitochondria. Growth on acetate is not affected, indicating that acetyl-CoA can be metabolised via the glyoxalate cycle in the cytoplasm. This is supported by the ability of various peroxisome mutants to grow on acetate but not on fatty acids. There are three classes of genes - those induced by acetate only; those induced by both acetate and fatty acids including *acuJ* and genes for the glyoxalate cycle and a very large number induced only by fatty acids including genes encoding beta-oxidation enzymes and peroxins. The transcription factor FacB, specific for acetate induction, is regulated by glucose repression and induction by acetyl-carnitine. FarA, FarB (orthologs of the highly conserved cutinase transcription factors) and ScfA, are required for fatty acid induction. FarA orthologs are also found in the hemi-ascomycetes, *Candida albicans*, *Debaryomyces hansenii* and *Yarrowia lipolytica*. Other carbon sources metabolised via the TCA cycle, like acetate, fatty acids and amino acids, require gluconeogenesis. Two transcription factors are required for induction of relevant enzymes in response to either malate or oxaloacetate.

Growth and developmental control in *A. nidulans* and *A. fumigatus*.

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Reproduction of fungi results in the formation of enormous numbers of spores that are efficient agents for genome protection, survival and propagation. Spores are also the primary agent for infecting hosts for many animal and/or plant pathogenic fungi. Asexual sporulation is a common reproductive mode for a diverse group of fungi. In some fungi asexual spore formation is intimately related with production of toxic secondary metabolites called mycotoxins, which cause various adverse health effects.

The genetically tractable fungus *Aspergillus nidulans* has served as an excellent model system for studying multicellular development and secondary metabolism. Overall goal of our research program is to understand the mechanisms underlying the regulation of vegetative growth and asexual development (conidiation) in *A. nidulans* and *Aspergillus fumigatus*. Via comparative functional studies of the four key *A. nidulans* regulators FadA (Ga), FlbA (RGS), FluG and BrlA in *A. fumigatus*, we found that 1) *A. fumigatus* and *A. nidulans* share conserved G protein and RGS-mediated growth signaling; 2) *A. fumigatus* may have the distinct and persistent upstream (*fluG*-level) regulatory mechanisms for activation of conidiation; and 3) BrlA, a key transcription factor necessary for conidiophore development in *A. nidulans*, is also essential for conidiation in *A. fumigatus*.

In addition to the comparative studies, we further dissected the regulation of spore formation in *A. nidulans*. Two distinct genetic screens have identified two key genes called *sfgA* and *vosA* that control initiation and completion of sporulation, respectively. Characterization of these genes revealed that both the beginning and the end of asexual spore formation require balanced activities of various positive and negative regulators. The genetically programmed instruction for the regulation of spore formation may explain how a fungus governs vegetative growth and reproduction appropriately. The detailed roles of these genes in developmental control are also presented.

The necrotrophic fungus, *Sclerotinia sclerotiorum* subverts host pathways by inducing apoptosis for disease development.

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Sclerotinia sclerotiorum is an extremely broad host range, economically important necrotrophic fungal plant pathogen. Effective pathogenesis by this fungus requires the secretion of oxalic acid (OA). Studies with OA mutants strongly suggest that oxalic acid is an essential pathogenicity determinant in *S. sclerotiorum* and is required for proper sclerotial development. Studies conducted to determine the mode of action of OA have indicated a multifunctional role for this organic acid. OA modulates ambient pH by acidification, which serves as a regulatory cue for processes linked to pathogenicity and differentiation including the activation of an OA induced mitogen activated protein kinase (SMK1) that is required for sclerotial development. Increases in cAMP levels impairs sclerotial formation and cAMP also inhibits the activation of *S. sclerotiorum* MAPK; thus cAMP-mediated sclerotial inhibition is modulated through MAPK. Cross-talk between these two pathways is mediated by the small G-protein, Rap-1.

Previous studies have shown that oxalate suppresses the oxidative burst from plants. Moreover, non-pathogenic OA-mutants, were unable to inhibit plant reactive oxygen species (ROS) induction. Thus a previously unrecognized function of oxalate is to suppress ROS generation and thereby compromise plant defense responses. ROS is not only involved with pathogenic (sclerotial) development in *Sclerotinia*, but is also critical for *Sclerotinia's* ability to sucessfully colonize host plant tissue. Evidence will be presented indicating that ROS is used as a signal to trigger host plant–cell death encoded pathways, resulting in apoptosis entirely for the benefit of the fungus. Thus, there is an apparent dual ROS regulatory scheme occuring; *Sclerotinia* suppresses plant defense via OA, while also generating ROS and inducing plant cell death pathways via OA. Thus, *Sclerotinia* modulates the redox environment during disease development.

An intelligent primitive eukaryote: environmental sensing in *Phycomyces*.

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The giant fruiting bodies, sporangiophores, of the Zygomycete *Phycomyces blakesleeanus* are characterized by their complex behavior. *Phycomyces* sporangiophores are aerial hyphae that grow out from the mycelium several centimeters into the air carrying at their top a small sphere filled with spores, the sporangium. Sporangiophore growth is governed by several environmental signals, including light, gravity, touch, wind, and the presence of nearby objects. These signals allow sporangiophore growth towards open air for efficient spore dispersal. Phototropism, sporangiophore growth towards light, has been investigated in detail. *Phycomyces* can react to a wide interval of light intensities, spanning ten orders of magnitude, and is able to respond to a small number of photons paralleling the sensitivity of the human eye. Two photoreceptors systems operating at different intensity ranges and a complex adaptation mechanism are used by *Phycomyces* to deal with the wide intensity range of light that can be perceived by the fungus. Mutants in *mad* genes have been isolated by their defect in phototropism. Some of the *mad* gene products are required for sporangiophore growth, but others are only required for phototropism and other light responses in this fungus. The discovery that the *madA* gene product is similar to *Neurospora* WC-1, a photoreceptor and transcription factor, suggests that the photoreception mechanism in *Phycomyces* may be related to that in *Neurospora*. A draft of the *Phycomyces* genome has been recently completed by the Joint Genome Institute (DoE, USA). The genome sequence will serve as a valuable resource to understand the molecular mechanisms regulating sporangiophore growth in *Phycomyces*.

Quorum sensing in bacterial-fungal interactions

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Candida albicans is responsible for a wide range of opportunistic fungal infections. Pathogenicity of *C. albicans* is largely dependent on its ability to undergo morphological interconversions between yeast and hyphal forms within the host. The control of these transitions is complex, and many environmental and chemical factors can influence the regulatory signaling cascades that govern *C. albicans* morphology. Recent studies have shown that *C. albicans* morphology is also influenced by bacterially-secreted products. In our studies, we have found that 3-oxo-C12 homoserine lactone, a signaling molecule produced by the Gram-negative bacterium *Pseudomonas aeruginosa*, is a potent inhibitor of hypha formation. Other molecules possess similar inhibitory activities, such as farnesol, a small molecule secreted by *C. albicans* itself. Using a combination of genetic and biochemical analyses, we have found that C12 compounds impact elements of the Ras1-cAMP signaling pathway, which positively regulates the yeast-to-hypha transition, by a mechanism separate from Ras1 activation. Furthermore, our data indicate that 3OC12HSL and farnesol impact the regulation of transcriptional responses other than those known to be involved in morphogenesis, suggesting that extracellular chemical signals may coordinately regulate multiple properties that are relevant in multicellular microbial communities.

Sex, time and evolution

Protein Folding, Environmental Contingency, and Evolution: Hsp90's Role in Fungal Drug Resistance.

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Hsp90 is an essential molecular chaperone in all eukaryotes that regulates the form and function of many key signal transducers. Hsp90 has a capacity to buffer the expression of genetic variation and to reveal it in response to environmental stress. As a capacitor for the storage and release of genetic variation in plants and flies, and likely other organisms, Hsp90 may play a role in evolution. In cancer cells, Hsp90 may promote evolution in a different way. It chaperones mutated cell regulators that are prone to misfolding but have activated oncogenic potential; rather than buffering the effects of new mutations, it allows them to have immediate phenotypic effects.

We exploited the emergence of fungal drug resistance as the ideal system for directly testing Hsp90's role in the evolution of new traits. In *Saccharomyces cerevisiae* and *Candida albicans*, we found that Hsp90 potentiated the evolution of drug resistance in a very different way. It enabled the phenotypic consequences of new mutations by chaperoning calcineurin, an unmutated regulator of cell signaling and key sensor of environmental stress. Hsp90's role in drug resistance was to enable crucial responses to specific stresses, including changes in the composition of cell membranes and cell walls. Pharmacological inhibitors of Hsp90 function abrogated drug resistance of diverse fungal pathogens, including *C. albicans* and *Aspergillus fumigatus*. Hsp90 inhibitors are now in clinical trials as anticancer agents, suggesting new therapeutic strategies for fungal infections. In nature, Hsp90 function can be overwhelmed by global protein misfolding due to stress, including elevated temperatures. We found that febrile temperatures reached in humans challenged by infection abrogated drug resistance, suggesting a specific clinical benefit of fever. Here we further explore the therapeutic potential of Hsp90 inhibitors and the molecular mechanisms by which Hsp90 acts to couple environmental contingency to the evolution and assimilation of new traits.

Comparative genomics of *Coccidioides* species.

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Coccidioides species are ascomycetous fungi found in hot, dry areas of the New World. They associate with endemic small mammals and have been the focus of research because they can cause life-threatening disease in otherwise healthy humans. Their virulence is reflected in their being the only fungi on the DHHS Select Agent list. Variation in gene sequences and microsatellite repeats have been used to recognize two species of *Coccidioides*, *C. immitis* and *C. posadasii*, and at least five populations therein. Through the efforts of the *Coccidioides* community, the Broad Institute and TIGR, there will be high-quality sequences available for at least two individuals from each of these five populations, and of a close relative, *Uncinocarpus reesii*. Already, genomes have been released for *U. reesii* and four *Coccidioides* individuals. This collection of genomes provides a rich resource for comparative genomics, conditional upon the quality of annotation. With annotated genomes for *Coccidioides* and *Uncinocarpus*, we now have searched for genes specific to *Coccidioides* or the outgroup, *Uncinocarpus*, for genes showing unusual rates of evolution, and for genes showing the effects of strong selection. Using microarrays, we have compared transcription profiling in two individuals from a single population and found that individual differences in gene transcription are significant. We will use transcription profiling of additional individuals to winnow the pool of genes showing significant changes in transcription in key points of the *Coccidioides* life cycle, especially the shift from saprobe to parasite. Genes that are identified as interesting from our comparative genomics, or from comparative transcription, or both, will become candidates for gene disruption or modification to test their role in pathogenicity using appropriate mammalian cell lines and a murine model of coccidioidomycosis.

Unwinding the Neurospora Circadian Clock

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Organisms from bacteria to humans use a circadian clock to control daily biochemical, physiological, and behavioral rhythms. We now have a firm understanding of the molecular oscillators that form the core of the circadian timing system. However, new data reveal that the circadian system is universally more complex than a single molecular feedback loop oscillator regulating all overt rhythmicity. Mounting evidence suggests that multiple oscillators comprise the *Neurospora crassa* clock. We have identified two *N. crassa* mutant strains that display circadian rhythms in the absence of the FRQ/WCC oscillator (FWO), considered to be the core of the fungal clock. These mutant strains uncover a novel circadian oscillator(s), which can function in cells that lack the FWO, but that is coupled to the FWO when the system is intact. Secondly, we have identified a new class of genes that cycle in mRNA accumulation in strains that lack FRQ. Characterization of one of these genes, *ccg-16,* has demonstrated that *ccg-16* mRNA rhythms are generated by a FRQ-less oscillator (FLO) that, similar to the FWO, requires functional WC proteins for activity. These data raise the possibility that the FWO and the FLO are coupled through the shared WC proteins. Critical questions that can now be addressed in *N. crassa*, and that are relevant to the organization of all clocks, including the human clock, are; what are the roles of multiple circadian oscillators in cells, and how do these oscillators communicate with each other to coordinately control rhythmicity?

Sex: How *Cryptococcus neoformans* Controls Itself.

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Cryptococcus neoformans is an opportunistic fungal pathogen that affects primarily immunocompromised individuals. Infections with *C. neoformans* are thought to be caused by spores, which can result from sexual development. During sexual development haploid **a** and alpha cells fuse and initiate a process controlled by the homeodomain proteins Sxi1alpha and Sxi2**a**. Our experiments are focused on determining the molecular mechanisms by which Sxi1alpha and Sxi2**a** control sexual development and, ultimately, spore production. Our hypothesis is that Sxi1alpha and Sxi2a control sexual development by directly regulating the transcription of key targets to specify the dikaryotic state**.** To identify targets of Sxi1alpha and Sxi2**a**, we are taking several integrated approaches, including using *C. neoformans* microarrays to identify genes regulated by Sxi1alpha and Sxi2**a**. Targets of interest are being tested for direct regulation by Sxi1alpha and/or Sxi2**a** using chromatin immunoprecipitations. Promoter sequences from both bioinformatic and microarray targets are being tested using in vitro DNA binding experiments with purified Sxi1alpha and Sxi2**a** proteins. Preliminary DNA binding studies show that the homeodomain regions of Sxi1alpha and Sxi2**a** bind specifically to the promoter sequences of many microarray

targets, including a putative homolog of the clampless (*CLP1*) gene. *SXI1alpha* and *SXI2**a*** are the first identified sexual cycle regulators in *C. neoformans*, and the characterization of the pathway they control will reveal how sexual development and spore formation occur in *C. neoformans*.

Origin and maintenance of virulence in human pathogenic fungi

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Human pathogenic fungi are acquired from other humans (e.g. *Candida* spp.) or the environment (e.g. *Cryptococcus neoformans*, *Histoplasma capsulatum*, etc). Human fungal infections are common, yet disease is rare, implying that humans posses a natural high resistance to fungal diseases. In fact, only a very small minority of fungal species have the capacity for mammalian virulence. This raises the fundamental question of whether pathogenic fungal species are different, and if so, why. In general, fungi acquired from other humans are normally part of the commensal flora and diseases caused by these fungi usually reflect a disruption of the host-microbe equilibrium. For example, candidiasis is often associated with antibiotic use, immunosuppression, or compromise integument and mucosal membranes. In contrast, human diseases caused by fungi acquired from the environment can occur in both normal or immunocompromised hosts, and the occurrence of these diseases often involve impaired immunity or unusual exposures with large inocula. For example, the likelihood of cryptococcosis increases dramatically in individuals with advanced HIV infection and both histoplasmosis and coccidioidomycosis can occur in normal individuals following infection with large inocula. Hence, whereas the origin of virulence for commensal fungi may be understood in the context of a disrupted host-microbe interaction, the origin and maintenance of virulence for the environmental fungi is more difficult to explain. Environmentally acquired fungal pathogens are free-living microbes that are adapted to specific ecologic sites and have no obvious needs for mammalian infection to procreate or survive. Most perplexing is the finding that soil organisms such as *C. neoformans* and *H. capsulatum* manifest sophistical intracellular virulence strategies that appear almost tailor made for mammalian virulence, despite having no requirement for these qualities. In recent years, an explanation for the origin of virulence among environmentally acquired fungal pathogens was proposed based on the emergence of certain traits as part of selection by environmental predators such as small animals, amoebae, and slime mold. According to this view, the emergence of virulence among the environmentally acquired pathogenic fungi is accidental, and involves the serendipitous selection of microbial characteristics that can function in mammalian factors by biotic and physical factors in the environment. The concept of 'accidental virulence' provides a useful construct for evaluating the origin, function, and maintenance of virulence factors that allows great freedom in approaching fundamental questions of microbial virulence. With these conceptual tools at hand, it is possible to take a different view of fungal virulence and its conceivable role in past extinctions such as the demise of dinosaurs.

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XXIV Fungal Genetics Conference at Asilomar

Poster Session Abstracts	Please cite abstracts as follows: Fungal Genet. Newsl. 54 (Suppl):Abstract#.

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CELL BIOLOGY

1. *Metarhizium anisopliae* perilipin homolog MPL1 regulates lipid metabolism, appressorial turgor pressure and virulence. Chengshu Wang and Raymond J. St. Leger. Department of Entomology, University of Maryland, College Park, MD, 20742, USA. stleger@umd.edu

Cells store lipids in droplets. Studies addressing the control of lipid-based energy homeostasis of mammals identified proteins of the PAT-domain family, such as perilipin that surround the lipid droplets. Factors that mediate lipid storage in fungi are still unknown. Here we describe a gene (*Mpl1*) in the insect pathogen *M. anisopliae* that has structural similarities to mammalian perilipins. Consistent with a role in lipid storage, Mpl1 is predominantly expressed when *M. anisopliae* is engaged in accumulating lipids and ectopically expressed GFP-tagged MPL1 localized to lipid droplets. Mutant *M. anisopliae* lacking MPL1 have thinner hyphae, fewer lipid droplets, particularly in appressoria and a decrease in total lipids. *Mpl1* therefore acts in a perilipin-like manner with an evolutionary conserved function in lipid metabolism. However, animal and fungal proteins have also been selected to cope with different tasks. Thus, turgor generation by Mpl1 appressoria is dramatically reduced indicating that lipid droplets are required for solute accumulation. This was linked with reduced ability to breach insect cuticle so that *Mpl1* is a pathogenicity determinant. Blast searches of fungal genomes revealed that perilipin homologs are found only in pezizomycotinal ascomycetes and occur as single copy genes. Expression of Mpl1 in yeast cells, a fungus that lacks a perilipin-like gene, blocked their ability to mobilize lipids during starvation conditions.

2. Characterization of AtmA and PrpA reveals a correlation between ROS and apical dominance in *Aspergillus nidulans*. Camile P. Semighini[1], Gustavo H. Goldman[2] and Steven D. Harris[1]. [1]Plant Pathology, University of Nebraska Lincoln; [2] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil Presenter's e-mail: csemighini2@unl.edu

The DNA damage response (DDR) is a protective mechanism that ensures the maintenance of genomic integrity. DDR is composed of many conserved proteins such as the Ataxia telangiectasia mutated (ATM) kinase and the Poly (ADP)- ribose polymerases (PARP). We are using the filamentous fungus *Aspergillus nidulans* as a model system and we believe that certain aspects of the DDR in this organism may reflect the unique organization of fungal hyphae. We have characterized the function of the *A. nidulans* ATM (AtmA) and PARP (PrpA) homologues. Besides their expected roles in DDR, those homologues also make an unexpected contribution to hyphal morphogenesis. In the absence of DNA damage, the delta*atmA* strain is defective in

polarized hyphal growth while the delta*prpA*$^{+/-}$ mutant produces an increased number of germ tubes per spore and displays an increase in the formation of lateral branches. Staining of a wild type strain with Nitro Blue Tetrazolium (NBT) revealed that a gradient of Reactive Oxygen Species (ROS) localize to the hyphal tip. NBT staining of delta*atmA* and delta*prpA*$^{+/-}$ strains correlated their hyphal morphology defect with disruption of ROS' specific localization. Our results suggest that ROS gradient may enforce apical dominance.

3. Roles of *Aspergillus nidulans cdc42* and *rac1* homologues in polarized growth. Aleksandra Virag and Steven Harris. Plant Science Initiative, N235 Beadle Center, University of Nebraska, Lincoln, NE 68588. E-mail: avirag2@unlnotes.unl.edu

In *Saccharomyces cerevisiae* the Rho GTPase Cdc42 is required for polarity establishment and maintenance. In contrast, Cdc42 homologues in filamentous Euascomycetes and Basidiomycetes do not appear to be important for polarity establishment, but rather for polarity maintenance and the establishment of secondary polarity axes-branches. The latter fungi also possess another Rho GTPase, Rac1, which is important for polarization but is absent from *S. cerevisiae*. Here we utilized the model Euascomycete *Aspergillus nidulans* to characterize the connection between Cdc42 and Rac1, and their presumed downstream components and effectors. Potential effectors include putative polarisome components (SpaA and SepA), as well as proteins required for microfilament and microtubule organization. Results suggest that Cdc42 and Rac1 have overlapping functions in polarity establishment. Preliminary genetic observations have identified, in addition to SpaA, other downstream effectors such as two distinct paxillin homologues. Based on our results we propose that Cdc42 and Rac1 cooperate in polarity-related processes.

4. Pheromone receptor Bar2 of the homobasidiomycete *Schizophyllum commune.* Susann Jezewski* & Erika Kothe. Friedrich-Schiller-University, Dept. of Microbiology, Neugasse 25, 07743 Jena, Germany; E-mail: Susann.Jezewski@uni-jena.de

The tetrapolar mating system of *Schizophyllum commune* allows easy discrimination of mating types and is associated with two genetic systems, which contain the *A* and *B* genes. Each *B* locus codes for one pheromone receptor, belonging to the family of G protein coupled seven transmembrane domain receptors, and several pheromones, small lipopeptide ligands. The interaction of a pheromone receptor and non-self ligands results in nuclear migration and clamp cell fusion, which are part of sexual development. There is no induction of *B* gene dependent sexual development by pheromones of self-specificity, of which several are encoded within each locus. The recognition of pheromones by multi-specific pheromone receptors and the distinction of self and non-self are necessary for a compatible mating process. The function of the pheromone/receptor system implies the localization of the receptor protein in the tip of growing hyphae or in clamp cells of *S. commune*. The visualization of the *Balpha2* receptor protein (Bar2) should be possible by a fusion of the intracellular C-terminus of Bar2 with fluorescent proteins, like GFP or DsRed. However, the transient process of clamp cell formation and the low expression of mating type genes make the determination of receptor molecules complicate. By means of real time PCR, a quantification of pheromone receptor mRNA will be possible in different mutant backgrounds under special conditions.

5. Does the single Dothideomycete MAT1-1-1 protein harbor domains for activities performed by multiple MAT1-1 proteins from Sordariomycetes? Banu Metin[1], Shun-Wen Lu[2], B. Gillian Turgeon[3]. [1]Department of Food Engineering, Middle East Technical University, 06531 Ankara, Turkey, e102950@metu.edu.tr. [2]Department of Plant Pathology, Cornell University, Ithaca, NY 14853, USA, swl2@cornell.edu [3]Department of Plant Pathology, Cornell University, Ithaca, NY 14853, USA, bgt1@cornell.edu

In filamentous ascomycetes, mating type loci exist as two alternate forms, MAT1-1 and MAT1-2, defining two mating types. While MAT1-2 contains a single gene with an HMG box domain in all ascomycetes examined so far, MAT1-1 shows differences. In Sordariomycetes, the MAT1-1 idiomorph contains three genes: MAT1-1-1 encoding a protein with an alpha 1 domain, MAT1-1-2 encoding a protein with an HPG domain, and MAT1-1-3 encoding a protein with an HMG box domain. In contrast, in Dothideomycetes, the MAT1-1 idiomorph harbors a single gene, which has been believed to contain only the alpha 1 domain. Careful comparison of the MAT1-1-1 and MAT1-2-1 protein sequences, however, reveals conserved motifs, including one resembling the HMG box sequence, in both proteins. To determine if this second HMG motif is functionally important in both MAT1-1-1 and MAT1-2-1, point mutations were made in MAT1-1-1 (W152A, G160F, V186A, and Y198*) and MAT1-2-1 (W138A, I158A, H170E) sequences of *Cochliobolus heterostrophus*. These mutated genes are being used to transform the *MAT*-deleted *C. heterostrophus* strain C4-41.7 and the strains will be tested for mating competence. Effects of the mutations on mating ability will address the question of whether the single MAT1-1-1 proteins of Dothideomycetes have evolved to carry out all activities provided by multiple MAT1-1 proteins of Sordariomycetes.

6. Analysis of a protein complex required for septation in the filamentous ascomycete *Ashbya gossypii*. Andrea Walther and Juergen Wendland. Carlsberg Laboratory, Yeast Biology, DK-2500 Valby, Copenhagen, Denmark

Hyphae of filamentous fungi are compartmentalized in regular intervals by septation. Key steps for septation are (i) the selection of a septal site, (ii) the formation of protein complexes at this site and (iii) actin-myosin ring constriction, which is accompanied

by chitin deposition. Previously we have analyzed the role of the *A. gossypii BUD3* gene in septum formation. Here we present evidence that Bud3 belongs to a protein complex at septal sites that involves septins and Bud4. We have analyzed the function of the septin encoding gene *CDC3* and the *A. gossypii* homolog of the *Saccharomyces cerevisiae BUD4* gene. Deletion of *CDC3* yields viable albeit slow growing mutants that exhibit a penetrant septation defect. Aberrant chitin deposition at random cortical positions is found in the *cdc3* mutant bearing similarity to the septation defect found in *bud3* strains. In contrast to *cdc3* strains, *bud3* hyphae also show correct septation. Mutant *bud3* strains are able to sporulate while *cdc3* strains do not form spores. Both, Cdc3 and Bud4 were localized at septal sites using GFP-tagged proteins. Using the *S. cerevisiae* two-hybrid system we established protein-protein interactions among these proteins. This establishes a network of proteins required for correct septum formation and further on may be used as a landmark to direct lateral branching.

7. Characterization of Protein *O*-mannosyltransferases in *Aspergillus nidulans*. Thanyanuch Kriangkripipat and Michelle Momany*. Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA tkriang@plantbio.uga.edu

Protein *O*-mannosyltransferases (PMTs) are found in bacteria, fungi, and animals but are not present in plants. In fungi, PMTs are divided into three subfamilies, PMT1, PMT2 and PMT4 and each species has 3-7 PMTs. *Aspergillus nidulans* possesses three PMTs, Pmt1, Pmt2 and Pmt4. Single *pmt* deletion mutants are viable. Each Δ*pmt* mutant exhibits different phenotypes when characterized by growth at different temperatures, morphology and sensitivity to chemicals disturbing cell wall synthesis. Double mutants show additive phenotypes. The Δ*pmt1* mutant has hyphal tip lysis and produces aberrant conidiophores at 42^{O}C. The Δ*pmt2* mutant cannot send out germ tubes at 42^{O}C. The

Δ*pmt4* mutant has swollen hyphae and produces aberrant conidiophores at 42^{O}C. The Δ*pmt1*Δ*pmt2* double mutant is viable and has additive phenotypes of Δ*pmt1* mutant and Δ*pmt2* mutant. The Δ*pmt2*Δ*pmt4* double mutant is viable but very sick and forms a microcolony only when an osmotic stabilizer is added to the medium. Lower temperatures and osmoticum can partially restore wildtype hyphal growth and conidiation of these Δ*pmt* mutants except for the Δ*pmt2*Δ*pmt4* double mutant. Our results suggest that protein *O*-mannosylation is important for cell wall integrity of *A. nidulans*.

8. The autophagy gene, *atgA*, is required for conidiophore development and starvation-induced hyphal growth in *Aspergillus fumigatus*. Daryl Richie, Michael Miley, David Askew . University of Cincinnati, Pathology and Laboratory Medicine

Autophagy is a highly conserved eukaryotic process in which cytoplasmic constituents are sequestered within a double membrane vesicle and delivered to the vacuole for the recycling of macromolecules. The purpose of this study was to determine the role of autophagy in the opportunistic human pathogen *Aspergillus fumigatus*. Autophagy was disrupted by deletion of the *A. fumigatus atgA* gene, which encodes the homolog of *Saccharomyces cerevisiae* Atg1p, a serine threonine kinase needed for induction of autophagy. The autophagy mutant showed abnormal conidiophore development and reduced conidiation, which could be corrected by supplementing the medium with additional nitrogen. When hyphal plugs of the autophagy mutant were transferred from rich medium to starvation medium they displayed reduced radial growth as compared to wild type. However, the addition zinc or manganese to starvation medium allowed the autophagy mutant to grow to the same degree as wild type. In rich medium, the autophagy mutant was hypersensitive to EDTA. However, the growth of the autophagy mutant in EDTA could also be restored to wild-type levels by the addition of excess zinc and manganese to the medium. Our results suggest that *A. fumigatus* uses autophagy to recycle intracellular stores of nitrogen to support optimal conidiation in a nitrogen limiting environment. Furthermore, our results suggest that autophagy in *A. fumigatus* allows for the recycling of limiting trace elements needed for optimal radial growth.

9. Cloning of the *eln1* gene essential for stipe elongation of *Coprinus cinereus*. Hajime Muraguchi[1], Naoki Takahashi[1], Sonoe O. Yanagi[1], Takashi Kamada[2]. [1]Department of Biotechnology, Akita Prefectural University, Akita, Japan. [2]Department of Biology, Okayama University, Okayama, Japan.

Coprinus cinereus fruit body exhibits remarkable stipe elongation in the final phase of development: the stipe elongates 8 times in one night. A dominant *elongationless1-1* (*eln1-1*) mutant defective in stipe elongation was first induced from the dikaryon. A recessive *elongationless1-2* (*eln1-2*) mutant was then induced from the monokaryotic fruiting strain, CopD5-12. During stipe elongation in the wild type, the cylindrical component cells of the stipe elongate with their diameter unchanged, resulting in slender stipe cells. In the mutants, however, the cylindrical cells increase in diameter and the rate of elongation is much reduced, resulting in globular stipe cells. Genetic analysis mapped the *eln1* locus 0.3 cM apart from *his5* on chromosome I. We transformed an *eln1-2* mutant with BACs assigned near *his5*, and found that two BAC clones, s2F7 and s14B9, have the complementing activity. We then defined the genomic region of the *eln1* gene within about 30 kb by testing subclones from s14B9 for their complementing activity. We are now trying to identify the *eln1* gene by testing PCR-amplified fragments from the 30-kb subclone.

10. A Rho1 Homologue From *Ustilago maydis*, Uro1, is Required for Normal Cell Growth. Zhanyang Yu[1], Cau D. Pham[1], Scott E. Gold[2], and Michael H. Perlin[1]. [1]University of Louisville, Department of Biology, Program on Disease Evolution, Louisville, Kentucky, USA and [2]University of Georgia, Department of Plant Pathology, Athens, Georgia, USA

In the pathogen of maize, *U. maydis*, a carefully orchestrated network of signaling is used to ensure that development proceeds in an orderly fashion. Uro1 is a homologue of the *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* Rho1 proteins, members of the family of Rho GTP-binding proteins that includes Cdc42 and Rac1. We have cloned and characterized the *uro1* gene encoding this *U. maydis* homologue. Over- expression of either wild type or constitutively-active mutant Uro1 caused a slight reduction in mating efficiency. A diploid strain in which a single copy of *uro1* was disrupted, similarly showed reduced "Fuz" reaction on charcoal agar relative to the wild type progenitor strain. This singly-disrupted diploid was unable to produce galls when injected into maize. To circumvent the likely lethality of disruption in haploid strains, the *uro1* gene was placed under control of the *crg* promoter, so as to allow its expression in arabinose media or its shut down in glucose. When *uro1* expression was shut off, cells failed to reproduce; however, they could recover if transferred to arabinose medium up to 72 h after the initial shut off by glucose. Several possible interactors of Uro1 were identified by yeast 2- hybrid, including Ump2 (an ammonium transporter), Smu1 (the Pak-like kinase involved in mating), a CDC24 homologue (a RhoGEF), and, quite interestingly, PTEN (phosphatase and tensin homologue deleted on chromosome 10), which acts as a tumor suppressor gene in mammalian cells. We propose that, among its several possible roles, Uro1 acts as an inhibitor of PTEN in *U. maydis*, and normally prevents the latter's action to promote arrest of cell division and/or apoptosis sequelae. We will provide additional experimental data which address this hypothesis.

11. **The Nuclear Migration Protein NUDF Associates with BNFA and NUDC at Spindle Pole Bodies in *Aspergillus nidulans*.** Kerstin Helmstaedt, Karen Meng, Silke Busch, Özgür Bayram, Oliver Valerius and Gerhard H. Braus. Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstr. 8, D-37077 Göttingen, Germany. Tel. +49-551-3919693, Fax +49-551-393820, khelmst@gwdg.de

In *Aspergillus nidulans*, nuclear division depends on microtubuli, the motor dynein and nuclear distribution proteins like NUDF. Applying tandem affinity purification, we isolated a unique NUDF-associated protein, which we named BNFA (Binding of NUDF). An *A. nidulans bnfA* deletion strain did not show a *nud* phenotype indicating that a protein with redundant function might exist. A GFP-BNFA fusion localized to spindle pole bodies (SPBs) throughout the cell cycle. This position was depended on NUDF, since in a *nudF6* strain BNFA localized mainly to dots in the cytoplasm. In a yeast two-hybrid screen using BNFA as bait, we found that BNFA is a dimer and that a link might exist to the septation signalling pathway. In a candidate approach, we analysed the putative NUDC-NUDF interaction in *A. nidulans*. Although NUDC-GFP alone was localized to immobile dots at the cortex, we found a direct interaction between NUDF and NUDC in yeast two-hybrid experiments, which depended on NUDF's WD40 domain. Applying bimolecular fluorescence complementation microscopy, we showed that *in vivo* NUDF and NUDC interact also at spindle pole bodies throughout the cell cycle and at immobile dots at the cortex.

12. **Regulation of hyphal morphogenesis and the DNA damage response by the *Aspergillus nidulans* ATM homolog, ATMA.** Iran Malavazi[1], Camile P. Semighini[2], Marcia Regina von Zeska Kress[1], Steven D. Harris[2], Gustavo H. Goldman[1]. [1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil; [2]Plant Science Initiative and Department of Plant Pathology, University of Nebraska, Lincoln, USA.

Ataxia telangiectasia (A-T) is an inherited disorder characterized by progressive loss of motor function and susceptibility to cancer. The most prominent clinical feature observed in A-T patients is the degeneration of Purkinje motor neurons. Numerous studies have emphasized the role of the affected gene product, ATM, in the regulation of the DNA damage response. However, in Purkinje cells, the bulk of ATM localizes to the cytoplasm and may play a role in vesicle trafficking. The nature of this function, and its involvement in the pathology underlying A-T, still remain unknown. Here we characterize the homolog of ATM (AtmA) in the filamentous fungus *Aspergillus nidulans*. In addition to its expected role in the DNA damage response, we find that AtmA is also required for polarized hyphal growth. We demonstrate that an *atmA* mutant has a severe defect in the control of nuclear proliferation and fails to generate a stable axis of hyphal polarity. Notably, cytoplasmic microtubules display aberrant cortical interactions at the hyphal tip. Our results suggest that AtmA regulates the function and/or localization of landmark proteins required for the formation of a polarity axis in *A. nidulans*. We propose that a similar function may contribute to the establishment of neuronal polarity. Financial Support: FAPESP and CNPq Brazil, Nebraska Research Foundation, USA

13. **Endocytic Pathway in Mushroom Development: Role of Le.Rab7 and Interacting Proteins.** M.T. Lee, Y.Y. Szeto, T.P. Ng, H.S. Kwan. The Chinese University of Hong Kong, Shatin, HKSAR

Endocytosis is a process through which substrates enter a cell without passing through the plasma membrane but via invagination of membrane and formation of intracellular vesicle. *Rab7* regulates the trafficking between early and late endosomes as well as late endosomes to lysosomes in the endocytic pathway. We have isolated *Le.Rab7*, from *Lentinula edodes*, that is highly homologous to *Rab7* (78% similarity). Two candidates, receptor for activated C kinase-1 (Le.RACK1) and Rab5 GTPase

(Le.Rab5) were isolated as interacting partners of Le.Rab7 through *in vivo* and *in vitro* protein interaction assays. *Le.Rab7* and *Le.RACK1* express strongest in primordium, while *Le.Rab5* expressed constitutively in all stages. *In situ* hybridization showed that the three transcripts were mainly localized at active growth regions of fruiting bodies. They strongly expressed at the outer region of trama cells and subhymenium in the hymenophore of gill tissue and at the prehymenophore in the ¡§eye organ¡¨ of young fruiting body. The protein localization of Le.Rab7 was also found in fruiting bodies. The existence of endocytosis in mycelium and gill tissue was shown by the internalization of endocytic dye, which co-localized with Le.Rab7 protein. It is the first report on the presence of endocytosis in basidiomycete. Le.Rab7, Le.Rab5 and Le.RACK1 may contribute to the production of basidiospores and the differentiation of mycelial cells to different cell types at the gill tissue.

14. **Analysis of two cell end marker proteins, TeaA and TeaR, in *Aspergillus nidulans*.** Norio Takeshita, Sven Konzack, Yuhei Higashitsuji and Reinhard Fischer. Applied Microbiology, University of Karlsruhe, Germany.

The interplay of the actin and the microtubule (MT) cytoskeleton in polarized growth of fungi has recently been revealed. In *Schizosaccharomyces pombe*, Tea1 is a key protein in this process. Tea1 is transported to the plus ends of MTs by the kinesin Tea2, and is delivered to cell ends by hitchhiking with the growing MTs. Mod5, which is posttranslationally modified by prenylation, anchors Tea1 at the cell ends. These three proteins were indentified by screening for polarity mutants. At the cell ends, Tea1 recruits formin which initiates actin assembly and the establishment of cell polarity. Tea1 and Tea2 homologues were identified in the *Aspergillus nidulans* genome (named TeaA and KipA), whereas Mod5 could not be identified due to sequence similarity. *kipA* mutants showed mislocalization of the Spitzenkorper and hence curved hyphae. GFP-KipA accumulated at the MT plus ends. *teaA* mutants showed a similar but not identical phenotype to *kipA* mutants. GFP-TeaA localized to one point in the apex of hyphal tips. To test whether the function of *S. pombe* Mod5 was conserved, we searched the *A. nidulans* genome for proteins with a C-terminal prenylation motif (CAAX). From 22 identified proteins one (536 amino acids) was likely to serve a TeaA-anchorage function. We named it TeaR. *teaR* mutants indeed displayed a phenotype similar to the *kipA* mutant. GFP-tagged TeaR localized to the membrane at hyphal tips. Their putative interactions will be analyzed.

15. **The *Aspergillus nidulans* putative kinase KfsA plays a role in septation and is required for efficient asexual spore formation.** Norio Takeshita, Anne Blumenstein, KayVienken and Reinhard Fischer Applied Microbiology, University of Karlsruhe, Germany

In eukaryotes cytokinesis has to be a well-controlled process to guaranty that each cell receives a nucleus. The process is well characterized in *Saccharomyces cerevisiae*, where a safeguard-machinery (MEN = mitotic exit network) has evolved to prevent cytokinesis before proper nuclear segregation. One important component is the kinase Kin4, which inhibits the MEN and prevents precautious cell division when the spindle position checkpoint is activated. In *Aspergillus nidulans*, nuclear division and cytokinesis are not coupled in hyphae, resulting in multinucleate compartments. In contrast, metulae, phialides and conidia are uninucleate. Here, we describe the role of a putative Kin4- related kinase, KfsA (kinase for septation) in the control of septum formation in *A. nidulans*. KfsA showed similarity to Kin4 only in the kinase domain but is conserved among filamentous fungi. *kfsA* mutants did not show any obvious phenotype in hyphal growth but produced less conidia. Conidiophores of *kfsA* mutants often contained several septa and a certain number of metulae harboured two nuclei and the corresponding phialides remained anucleate. The latter result suggests a septum-control mechanism of KfsA during nuclear distribution in the yeast-like growth of metulae, which guarantees that cytokinesis only occurs if both cells contain a nucleus. A GFP-KfsA fusion protein localized to septa in hyphae and the conidiophore and to the cortex of hyphae.

16. **Nuclear anarchy: asynchronous nuclear division in *Ashbya gossypii.*** Amy S. Gladfelter. Dartmouth College, Department of Biological Sciences amy.gladfelter@dartmouth.edu

Multinucleated cells are essential for disease processes such as tumorigenesis and fungal infection, but little is known about how the unique organization of multinucleated cells influences the control of mitosis. We have found that nuclei divide asynchronously in the filamentous ascomycete *Ashbya gossypii*. In these cells, neighboring nuclei are in different stages of the nuclear division cycle despite being only a few micrometers apart and bathed in the same cytoplasm. Similar asynchronous nuclear division also has been observed in multinucleated tumors and other filamentous fungi, but how independent nuclear behavior is established in the context of a common cytoplasm remains to be discovered. Using in vivo time-lapse microscopy, automated image analysis, and molecular genetic approaches we are currently testing two main hypotheses to determine when and how nuclear asynchrony develops: 1.) Nuclear asynchrony emerges in the G1/S transition due to the unequal distribution of cell cycle regulators among nuclei and 2.) The unequal localization of cell cycle regulators among nuclei arises due to differences in Spindle Pole Body age and/or Nuclear Pore Complexes between neighboring nuclei. With these studies we are identifying mechanisms regulating the G1/S transition and delineating how nuclear asymmetries are established in multinucleated cells.

17. **CRE-1, a regulator of catabolite repression is involved in determining PKA-dependent polarity of *N. crassa*** Carmit Ziv, Rena Gorovits and Oded Yarden. Dept. of Plant Pathology and Microbiology, The Otto Warburg Minerva Center for

Agricultural Biotechnology Faculty of Agricultural, Food and Environmental Quality Sciences The Hebrew University of Jerusalem Rehovot 76100, Israel

Polar hyphal elongation is a complex event in fungal growth and is affected by multiple factors. The *Neurospora crassa mcb* gene encodes a regulatory subunit of the cAMP-dependent protein kinase A (PKA). We have identified a 19bp deletion at the 5' UTR of *mcb* temperature-sensitive allele which we propose impairs transcript integrity of the regulatory subunit and thus may result in increase PKA activity and a complete loss of growth polarity at restrictive temperatures. As a link between the PKA pathway and carbon source sensing has been established in *Aspergillus* and yeasts, we tested the effect of different carbon sources on *mcb* morphology. Surprisingly, when grown in the presence of fructose, but not in either glucose or sucrose, the typical bulbous *mcb* phenotype was completely suppressed. As glucose and fructose equally support *N. crassa* wild-type growth we concluded that the differential effect on *mcb* involves a regulatory, rather than a metabolic, mechanism. To determine the involvement of carbon catabolite repression (CCR) in determining PKA-dependent polarity, we inactivated a key CCR regulator, *cre- 1*. The delta-*cre-1* strain showed reduced growth on glucose vs. fructose, in addition to altered hyphal morphology and impaired CCR, as evident by changes in *inv* and *adh-1* expression and amylase and beta- gal activity. The deletion of *cre-1* in an *mcb* background partially restored polarity when grown on glucose, indicating that downstream CRE-1-regulated elements can compensate for polarity defects caused by mis-regulated PKA activity. These results suggest a role for the CRE-1-mediated cellular response to carbon source in determining PKA- dependent polar growth in *N. crassa*.

18. Altering COT1 kinase phosphorylation sites affects its function in *N. crassa* Carmit Ziv, Rena Gorovits and Oded Yarden. Dept. of Plant Pathology and Microbiology, The Otto Warburg Minerva Center for Agricultural Biotechnology Faculty of Agricultural, Food and Environmental Quality Sciences The Hebrew University of Jerusalem Rehovot 76100, Israel

The *Neurospora crassa* COT1 kinase is the founding member of the conserved NDR protein kinase family. Defects in *cot-1* confer a highly pleotropic phenotype typified by a restricted, colonial, growth. *In vitro* analysis of mammalian NDR demonstrated the involvement of conserved phosphorylation sites in the regulation of NDR kinase activity. Using antibodies against the phosphorylated human NDR protein we detected the presence of the two conserved phosphorylation sites in *N. crassa* COT1: Ser-419, a putative MOB2-stimulated autophosphorylated site and Thr- 591, a putative Ste20-like kinase-phosphorylated site required for NDR activity. To determine the significance of the phosphorylation states of COT1 on cell morphology *in vivo*, we analyzed strains harboring a *5xmyc::cot- 1* fusion allele at the *cot-1* locus, in which these conserved regulatory residues have been substituted by either Glu or Ala to mimic the phosphorylated and unphosphorylated states, respectively. The resulting 4 strains (designated S419E, S419A, T591E and T519A) were viable, yet some presented altered morphology. The importance of Ser-419 was evident by a reduced growth rate of both S419E and S419A strains. In addition S419A exhibited a dramatic abnormal, hyper-branched, morphology and produced a *ropy*-like colony. Altering Thr-591 resulted in a milder effect; strain T591E showed reduced aerial hyphae and enhanced carotenoid production, while T591A displayed a reduced growth rate. Determining COT1 kinase activity levels, localization and interaction with additional proteins in these strains will help elucidate the *in vivo* role of phosphorylation in governing specific functions of COT1.

19. **The BEM46-like protein is essential for hyphal growth from ascospores of *Neurospora crassa* and targeted to the ER**. Mercker M, Alves S, Weiland N, and F. Kempken. Botanisches Institut, Christian-Albrechts-Universität zu Kiel, Olshausenstr. 40, 24098 Kiel, Germany; fkempken@bot.uni-kiel.de

BEM46 proteins are evolutionary conserved members of the α/β-hydrolase super family. However, to date, their exact role remains unknown. Therefore, to better understand the cellular role of BEM46 and its homologs, we used the model organism *Neurospora crassa* in conjunction with *bem46 RNAi*, overexpression vectors, and repeat induced point (RIP) mutation analyses. These studies clearly demonstrated that BEM46 is required for cell-type- specific hyphal growth and indicate a role of BEM46 in maintaining polarity. For example, vegetative hyphae, perithecia, and ascospores develop normally, but hyphae germinating from ascospores exhibit a strong loss-of-polarity phenotype. Moreover, we found that the BEM46 protein is targeted to the ER, and localizes at or close to the plasma membrane. BEM46 thus being a new ER marker for filamentous fungi, and the first for the model organism *Neurospora crassa*. Thus our data suggest BEM46 plays a role in a signal transduction pathway that is involved in determining or maintaining cell-type-specific polarity. This finding also implies a higher degree of differentiation of fungal hyphae than currently expected.

20. Functional analysis of histidine-containing phosphotransmitter gene *ypdA* in *Aspergillus nidulans*. Natsuko Sato, Kentaro Furukawa, Tomonori Fujioka, Osamu Mizutani, and Keietsu Abe. Molecular and Cell Biology, Tohoku University, Sendai, Japan.

The high-osmolarity glycerol (HOG) response pathway responding to osmotic stimuli has been well studied in *Saccharomyces cerevisiae*. Sln1p-Ypd1p-Ssk1p proteins organize a two-component signalling (TCS) unit in the upstream of the HOG pathway, and negatively regulate the downstream Hog1p mitogen-activated protein kinase (MAPK) cascade. We previously revealed that a filamentous fungus *Aspergillus nidulans* possesses all counterparts of the components of *S. cerevisiae* HOG pathway. Deletion of

Ypd1p, the TCS histidine-containing phosphotransmitter of *S. cerevisiae*, is known to cause lethality because of constitutive activation of Hog1 MAPK. While, *S. cerevisiae* possesses only one set of TCS unit consisted of Sln1p and Ypd1p, *A. nidulans* has been predicted to have 15 histidine kinases and some of them are thought to interact with the unique YpdA. Thus, the TCS pathway of *A. nidulans* might be more complex and robust than that of yeast . In addition, *YPD1* is essential in *S. cervisiae* but not in *Shizosaccharomyces pombe*. In the present study, in order to examine in vivo functionality of *A. nidulans ypdA* gene, we constructed an *ypdA* delta strain conditionally expressing the *ypdA* gene under the control of *A. nidulans alcA* promoter and investigated its phenotypes under the *ypdA*-repressed condition. Downregulation of *ypdA* transcription caused sever growth inhibition. We observed a constitutive phosphorylation of HogA MAPK in *A. nidulans ypdA* delta. These results suggest that YpdA is a essential component of the upstream of *A. nidulans* HOG (AnHOG) pathway, and the growth inhibition caused by *ypdA* delta would be attributed to disorder of signalling through the AnHOG pathway.

21. **Calcium plays an essential role in the toxicity of the *Penicillium chrysogenum* antifungal protein PAF in *Aspergillus nidulans*.** Ulrike Binder[1], Sairah Saeed[2], Andrea Eigentler[1], Diana Bartelt[2], Florentine Marx[1]. [1]Biocenter, Division of Molecular Biology, Innsbruck Medical University, Fritz-Pregl Strasse 3, A-6020 Innsbruck, Austria [2]Dept. of Biological Sciences, Institute for Biotechnology, St. John's University, 8000 Utopia Parkway, Queens, NY 11439

The small, basic and cysteine-rich protein PAF is secreted by *Penicillium chrysogenum* and exhibits growth inhibitory activity against various members of filamentous ascomycetes including human and plant pathogens. We are interested in the elucidation of the mechanism of action of this antifungal protein. Previous investigations showed that PAF toxicity is related to plasma membrane hyperpolarization and the activation of ion channels. We hypothesize that PAF toxicity results at least in part in the perturbation of the calcium homeostasis. In this study we show, that minimal concentrations of calcium neutralize PAF activity. Furthermore, we detected a PAF–induced elevation of the intracellular calcium concentration in an *Aspergillus nidulans* strain that expresses the calcium sensitive photoprotein aequorin. This increase in intracellular calcium concentration was abrogated by the addition of the extracellular calcium chelator EGTA, which indicates that an influx of extracellular calcium is responsible for this effect. Verapamil, an L-type calcium channel blocker, positively influenced the growth of PAF–exposed *A. nidulans*. Further studies are presently carried out to characterize the role of calcium homeostasis in the toxicity of PAF in detail. This work is supported by the Austrian National Bank and the Austrian Science Foundation to F.M. and in part by NIGMS R15GM077345 to DB.

22. **Functional Comparison of CDC42 and RAC1 in the Dimorphic Fungus *Ustilago maydis*.** Andrea Hlubek and Michael Bölker. Fachbereich Biologie, Universität Marburg, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany; email: ahlubek@web.de

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 form, GTPase activating proteins (GAPs) stimulate the GTPase activity, thereby leading to the formation of the inactive state. In the dimorphic fungus *U. maydis* the GTPases Cdc42 and Rac1 are highly similar but trigger distinct cellular responses. We could show that *U. maydis* Cdc42, but not Rac1, complements the lethal phenotype of the temperature sensitive *cdc42-1* allele in *S. 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 Cdc42 and Rac1. In vivo and in vitro experiments let us hypothesize that specificity is regulated by interaction with the GEFs, which we are currently characterizing. Another important spatial regulator of GTPases is the Guanine dissociation inhibitor RhoGDI (Rdi1), which is important for proper localization of GTPases and therefore necessary for distinct cellular responses. Interestingly, the deletion of *rdi1* in *U. maydis* interferes with filament formation induced by Rac1 overexpression.

23. **The Role of UncA, an Unc104-related kinesin motor protein, in *Aspergillus nidulans*.** Nadine Zekert, Daniel Veith and Reinhard Fischer. Applied Microbiology, University of Karlsruhe, Germany.

We have previously shown that in *Aspergillus nidulans* conventional kinesin, KinA, is required for fast hyphal growth and the Kip2 family motor, KipA, for the establishment and maintenance of hyphal polarity. Here, we characterize UncA, an Unc-104 related motor protein. UncA (1631 amino acids) contains a motor domain, a forkhead association domain and a PH domain. A homologue of this motor was shown to be required for mitochondrial motility along microtubules in *Neurospora crassa*. However, in *A. nidulans* mitochondrial movement rather depends on actin. Deletion of *uncA* in *A. nidulans* caused slower hyphal extension with more branching, suggesting a role in polar growth. The fact that *uncA* deletion was not lethal suggested redundant functions of other kinesins. KinA was a good candidate for this, but surprisingly, a *kinA-uncA* double mutant was viable and displayed the same branching phenotype as the *uncA* mutant while its growth phenotype resemble the *kinA* mutant. UncA- GFP fusion displayed a spot-like distribution in the cytoplasm. The spots showed rapid bidirectional movement. The introduction of a rigor-mutation into *uncA* inhibited this movement and led to a rod-like staining pattern. Experiments to show whether these rods are microtubules or e.g. mitochondria are under way. A second Unc104-related motor was additionally found in the genome and named UncB (671 amino acids). The function will be characterized by deletion and subcellular localization studies.

24. ***Aspergillus fumigatus rasA*: a non-essential Ras homolog that regulates germination, mitosis and hyphal morphology.** Jarrod Fortwendel and Judith Rhodes. University of Cincinnati, Cincinnati, Ohio, USA.

Ras subfamily genes are known to control growth and differentiation in a wide variety of microorganisms. The majority of Ras homologs in pathogenic organisms act as molecular switches that regulate morphogenesis and, in some cases, virulence. The *A. fumigatus* genome contains two members of the prototypical Ras family of small GTPases, *rasA* and *rasB*. Of these Ras sub-family genes, *rasA* shares 98% homology to the essential *rasA* gene of *Aspergillus nidulans*. In this study, we report that deletion of *A. fumigatus rasA* caused delayed germination, nearly absent radial outgrowth, and decreased hyphal mass. Growth of the *rasA* deletion mutant on solid agar revealed an extremely compact colony morphology with markedly decreased conidiation. Although conidiation was reduced and delayed, the conidial viability is at wild-type levels. Growth in liquid media caused development of meandering hyphae with increased diameter, when compared to wild type. The *rasA* deletion mutant was resistant to protoplast preparation, even when using twice the levels of cell wall digestion enzymes that are sufficient for the wild type organism. Normal mitotic events in the mutant were also disturbed, as staining with propidium iodide revealed many small nuclei that were irregularly positioned throughout the hyphae. These data show that *rasA* is important to a wide range of cellular processes during the complex growth patterns of the important human pathogen *A. fumigatus.*

25. **A PTS2 peroxisomal targeting sequence in ApsB is required for functioning of non-nuclear MTOCs in *Aspergillus nidulans*.** Daniel Veith, Nadine Zekert, Rüdiger Suelmann and Reinhard Fischer. Applied Microbiology, University of Karlsruhe, Germany.

Peroxisomes are a diverse class of organelles involved in different physiological processes in eukaryotic cells. A special class of peroxisomes is represented by the fungal Woronin body, in which one protein, Hex1 in *Neurospora crassa*, forms a hexagonal crystal and serves a structural function in plugging septal pores after damage of hyphae. Here, we suggest a new class of peroxisomes, which is involved in microtubule formation. In *Aspergillus nidulans*, septum- associated microtubule-organizing centres (MTOCs) are polymerizing cytoplasmic microtubules in addition to MTOCs at the spindle pole bodies (SPBs). Previously, we identified a novel MTOC-associated protein in *A. nidulans*, ApsB, whose absence affected microtubule formation from septal MTOCs more than from SPBs, suggesting different organization of the two protein complexes. In a yeast-two-hybrid screening, we identified the peroxisomal protein HexA as an interacting protein of ApsB and discovered that ApsB is targeted to peroxisomes via a PTS2 peroxisomal targeting sequence or by a piggy-bag mechanism together with HexA. Peroxisomal localization of ApsB was necessary for normal function of septal MTOCs. ApsB translocated into Woronin bodies was not active, suggesting two distinct populations of septal peroxisomes, the Woronin bodies and the ones required for MTOC activity.

26. **Evolution of the mechanisms that regulate cellular morphogenesis in fungi: a comparative genomics approach.** Haoyu Si, Kaimei Xu, and Steven D. Harris*. Plant Science Initiative and Department of Plant Pathology, University of Nebraska, Lincoln, NE, 68588-0660. *Contact: Sharri1@unlnotes.unl.edu

The defining feature of fungal cells is polarized growth, whereby cell wall deposition is confined to a discrete location on the cell surface. The annotation of multiple fungal genome sequences has revealed that the signaling modules and morphogenetic machinery involved in polarized growth are largely conserved across the fungal kingdom. Nevertheless, fungal cells exhibit a diverse variety of shapes that are largely based on two growth patterns: hyphae and yeast. We suggest that these different patterns reflect variation in the mechanisms that spatially and temporally regulate cellular morphogenesis. To test our hypothesis, we are using a comparative genomics approach to examine the functional evolution of the regulatory modules that specify budding patterns in *S. cerevisiae*. The landmarks that specify the axial budding pattern (Bud3, Bud4, and Axl2) are weakly conserved in the Pezizamycotina. We are in the process of deleting and charactering these homologues in *A. nidulans*, *Neurospora crassa*, and *Fusarium graminearum*. Our preliminary results show that Bud3 and Bud4 are required for septum formation and, suggest that Bud3 may serve as a GEF (guanine nucleotide exchange factor) for the GTPase Rho4. We propose that the ancestral function of the axial markers may be the regulation of septin organization, which, upon the loss of hyphal growth in *S. cerevisiae*, allowed them to be co-opted into the module that regulates axial budding. By contrast, only two of the landmarks that specify the bipolar budding pattern (Rax1 and Rax2) are conserved in the Pezizamycotina. Moreover, our deletion analyses have failed to uncover an obvious role for these homologues in hyphal morphogenesis.

27. **Survival following exposure to hydrogen peroxide (H_2O_2) is developmental stage- dependent in *Aspergillus nidulans*.** Sairah Saeed and Diana Bartelt. Dept. of Biological Sci., St. John's University, Queens, NY 11439

Among the earliest antimicrobial defense mechanisms is the release of H_2O_2 formed during the respiratory burst into the phagosome during the degranulation process. *Aspergillus sp.* are opportunistic pathogens, causing a high rate of mortality in immunosuppressed patients. The ability to survive exposure to hydrogen peroxide is important in the onset of invasive *Aspergillosis* . We have found that survival of *A. nidulans* after exposure to hydrogen peroxide is dependent upon the stage of development, the density of the culture and the concentration of H_2O_2. Exposure of 10 hr. mycelial cultures to 10 mM peroxide causes greater than 90 percent killing while greater than 90 percent of cells in an 18 culture at the same density survive. Stability

of H_2O_2 in the medium is also dependent upon the density of the culture, the stage of development and H_2O_2 concentration. There appears to be a positive correlation between viability and the ability of the culture to destroy H_2O_2. The induction of catalase and/or peroxidase activity following exposure to H_2O_2 is examined. [Supported by NIGMS R15GM077345-01A1].

28. **Genetic and molecular analysis of a temperature-sensitive mutant *un-17* carrying a mutation in the poly (A) polymerase gene in *Neurospora crassa*.** S. Tanaka, N. Takayanagi, K. Murasawa, C. Ishii, and H. Inoue. Lab. of Genet., Fac. of Sci., Saitama Univ., Saitama, Japan.

un-17 mutant was isolated as a temperature-sensitive (ts) mutant in *Neurospora crassa*. This mutant shows several interesting phenotypes. In restriction temperature (37 C°), the growth immediately stops and the cells are going to die. This death is suppressed by adding of cycloheximide or conditions repressing growth. Even in permissive temperature, it shows female sterility and is deficient in production of extracellular superoxide dismutase SOD4. To know the function of the *un-17* gene, we tried to clone the gene. We mapped a position of the mutation to right of linkage group III, and searched a DNA fragment which complements the ts phenotype of *un- 17* mutant from a *N. crassa* genome library. The cloned gene had homology to yeast poly (A) polymerase (PAP), and the *PAP*-homolog gene of *un-17* had a mutation of one base substitution. New strains which express a recombinant *PAP* derived from either wild-type or *un-17* strain were constructed and analyzed. The recombinant PAP of wild-type indicated the activity of PAP, and *un-17* strains which were induced a constitutive expression of wild-type *PAP* recovered mutant phenotypes of original *un-17* strains. These data suggest that *un-17* encodes the essential PAP gene.

29. **Two-component response regulators, ChSsk1p and ChSkn7p, additively regulate high-osmolarity adaptation and fungicide sensitivity in *Cochliobolus heterostrophus*.** Kosuke Izumitsu. Kyoto University, Graduate School of Agriculture, Kyoto, Japan

Filamentous ascomycetous fungi possess many histidine kinases and two conserved response regulators, Ssk1p and Skn7p, in their two-component signaling systems. We previously reported that the fungi-unique group III histidine kinase regulates high-osmolarity adaptation and iprodione/fludioxonil fungicide sensitivity by controlling the phosphorylation of Hog1-type mitogen-activated protein kinase (MAPK) in filamentous ascomycetes. Here, we have characterized the response regulator genes ChSsk1 and ChSkn7 in the Southern corn leaf bright fungus *Cochliobolus heterostrophus*. Both Chssk1- and Chskn7-disrupted mutants showed little sensitivity to high-osmolarity stress and moderate resistance to the iprodione/fludioxonil fungicides. The phosphorylation of Hog1-type MAPK BmHog1p induced by high-osmolarity stress and fungicide treatments was only regulated by ChSsk1p, indicating that ChSkn7p has roles in high-osmolarity adaptation and fungicide sensitivity that are independent from the activation of BmHog1p. The ChSsk1/ChSkn7 double mutants clearly showed higher sensitivity to osmolar stress and higher resistance to fungicides than the single mutants. The dose responses of the double mutants fit well with those of the group III histidine kinase-deficient strain. These results suggest that in filamentous ascomycetes, the Ssk1-type and Skn7-type response regulators control high-osmolarity adaptation and fungicide sensitivity additively with differential mechanisms under the regulation of the group III histidine kinase. This study provides evidence that filamentous fungi have a unique two-component signaling system that is different from yeast and is responsible for high-osmolarity adaptation and fungicide sensitivity.

30. **Functional topology of the growing hyphal tip.** Michael Köhli[1], Philipp Knechtle[1], Virginie Galati[1], Kamila Boudier[1], Robert Roberson[2] and Peter Philippsen[1]. [1] Biozentrum, University of Basel, Klingelbergstrasse 50/70, 4056 Basel, Switzerland. [2] School of Life Sciences, Arizona State University, Tempe AZ 85287, USA.

Growth of fungal hyphae depends on initial definition of a polarity axis, tip-directed intracellular transport and efficient polarized secretion. A complex protein network whose components are conserved in eukaryotes coordinates these processes. While molecular data have become available only recently, a hallmark of polarized growth in filamentous fungi was described long time ago: the Spitzenkörper, a vesicle-based, dynamic structure that is found in the tip of growing hyphae. In order to identify proteins colocalizing with the Spitzenkörper, we monitored GFP-fusions to 14 polarity factors of *Ashbya gossypii*. Interestingly, in several cases, a given GFP-fusion showed localization patterns that differed between hyphae of the same mycelium, even if these hyphae were spaced less than 50 µm apart from each other. We show that these differences in localization correlate with hyphal growth speed. In slow growing hyphae, the investigated polarity factors mostly localized to the apical cortex independently of the factor's identity. Contrary, in fast growing hyphae, at least four functional zones defined by distinct sets of polarity factors were observed: the Spitzenkörper, the central apical cortex, the entire apical cortex and the extended apical cortex. The putative polarisome components *Ag*Spa2, *Ag*Pea2 and *Ag*Bni1, the Rab-GTPase *Ag*Sec4 and the exocyst components *Ag*Sec3 and *Ag*Exo70 were found to localize to the Spitzenkörper. These findings suggest that the Spitzenkörper is directly involved in regulation of the actin cytoskeleton and secretion but not in polarity maintenance or establishment *per se*.

31. **Variation in nuclear DNA content and chromosomes among parent isolates and progeny in *Phytophthora infestans*.** Mursel Catal, Pavani Tumbalam, Willie Kirk, Gerard Adams. Department of Plant Pathology, Michigan State University, East Lansing, MI 48824.

The DNA content of nuclei of various isolates of *P. infestans* and progeny from controlled matings was assessed by laser flow cytometry. Variation in size of chromosomes among isolates was evaluated with Southern hybridizations, and some virulence genes were located on chromosome fragments. Progeny varied in virulence, race, mating type, cold tolerance and genetic markers. In each controlled mating a small proportion of progeny were unusual, showing secondary homothallism, loss of virulence, and the combined markers of both parents. Variation in DNA content of nuclei among parents and, more interestingly, among the unusual progeny are presented and discussed in relation to chromosome patterns and ploidy.

32. **The *Aspergillus nidulans* nuclear pore complex protein An-Nup-2 plays a novel role in mitosis but is not essential for nuclear transport.** Sarine Markossian and Stephen A. Osmani. The Ohio State University, Columbus, Ohio, USA. markossian.1@osu.edu

The nuclear pore complex (NPC) regulates nuclear trafficking and is composed of ~30 subunits called nucleoporins (Nups). In yeast, Nup2p has been shown to facilitate nuclear transport. Unlike yeast Nup2p, *Aspergillus nidulans* Nup2 (An-Nup2) localizes to chromatin during mitosis but to the NPC during interphase. This indicates An-Nup2 may play a role during mitosis. An-*nup2* is essential and its deletion causes mitotic defects. We therefore speculate that the localization of An-Nup2 to mitotic chromatin is important for mitosis. To test this hypothesis, a domain study was performed to define the An-Nup2 domain responsible for its mitotic translocation to chromatin and an antibody was generated against An-Nup2. A domain spanning from aa 400 to aa 1200, which encompasses a basic stretch of amino acids, a coiled coil region, and two potential nuclear localization sequences (NLS), is sufficient to locate An-Nup2-GFP to the NPC during interphase and to DNA during mitosis. The An-Nup2 antibody was used for immunofluorescence and successfully stained the nuclear periphery during interphase and chromatin during mitosis confirming the An-Nup2-GFP localization. Most importantly, the heterokaryon rescue technique was used to define if nuclear transport and/or mitosis is defective without An-Nup2. An-Nup2 deleted cells were not deficient in nuclear transport of NLS-dsRed suggesting that the lethality caused by An-Nup2 deletion is due to mitotic defects and not nuclear transport defects. In conclusion, there is emerging evidence that the localization of An-Nup2 to mitotic chromatin is essential for mitosis although An-Nup2 may not be essential for nuclear transport.

33. **Cell biology of the mode of action on *Neurospora crassa* of an antifungal agent from *Hypocrea atroviridis*.** Verena Seidl[1, 2], Christian P. Kubicek[2] and Nick D. Read [1]. [1]Institute of Cell Biology, University of Edinburgh, UK, [2]Institute of Chemical Engineering, Vienna University of Technology, Austria.

The well characterized model system, *Neurospora crassa*, was used as a host for the necrotrophic mycoparasite *Hypocrea atroviridis* (*Trichoderma atroviride*) to get new insights into the mechanisms underlying mycoparasitism by *Hypocrea/Trichoderma* spp. Although mycoparasitic *Hypocrea/Trichoderma* spp. have a wide host range, their interactions are mostly studied with plant pathogens, and many aspects of the mycoparasitic process are not well understood. *Hypocrea/Trichoderma* spp. produce highly effective antifungal compounds and antibiotics, but very little is known about their specific modes of action. *H. atroviridis* displayed antagonism against *N. crassa* in plate confrontation assays. Before making contact, *N. crassa* hyphae showed a severely stressed phenotype characterized by slow, bud-like growth and hyperbranching. Spore germination was also strongly inhibited. To characterize the stress response in more detail we are studying the effect of the inhibitory compound on various subcellular processes, including Spitzenkörper formation, endocytosis, membrane potential and mitochondrial activity. The inhibitory compound is constitutively secreted by *H. atroviridis* independent of the presence of a host. It was isolated and purified and could be shown to be a small, non-volatile organic molecule and it is currently being characterized by mass spectrometry.

34. **Identification of novel genes involved in hyphal fusion in filamentous fungi.** Alex Lichius and Nick D. Read. Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, UK. (A.Lichius@sms.ed.ac.uk)

Vegetative hyphal fusion is essential for the establishment of an interconnected colony network, and is a key feature of the filamentous fungi. A small number of proteins involved in regulating hyphal fusion have been identifed in *Neurospora crassa*. Hyphal fusion and appressorium formation in fungal pathogens share a number of features in common, including adhesion, cell swelling, secretion of cell wall degrading enzymes, penetration and MAP kinase signalling. We are asking the question: how much does the machinery involved in appressorium formation share in common with that involved in hyphal fusion? To answer this question and identify new proteins involved in these processes, we are screening 42 knockout mutants of genes in *N. crassa* which are orthologs of genes encoding proteins involved in appressorium formation and/or are pathogenicity determinants in the rice blast fungus, *Magnaporthe oryzae*. Processes in which these *Magnaporthe* proteins play a role include surface recognition, cell adhesion, appressorium induction, appressorium development, and host cell penetration. Conidial anastomosis tube (CAT) fusion is being used as simple and convenient experimental system for this genetic screen. We are also assessing hyphal fusion in mature colonies and use a wide range of live-cell analytical techniques to characterize the mutants. Acknowledgements: AL is funded by the School of Biological Sciences studentship of the University of Edinburgh.

35. **A Cytoplasmic Nuclear Remnant is involved in the Segregation of Nucleolar proteins in *Aspergillus nidulans*.** Leena Ukil, Colin De Souza, Hui-Lin Liu and Stephen A. Osmani, Department of Molecular Genetics, The Ohio State University, 484 W 12th Ave, Columbus, OH 43210, Email: leenaukil@yahoo.com

The nucleolus is a prominent nuclear structure whose mitotic segregation is poorly understood. During yeast mitosis the nucleolus segregates intact with rDNA. In contrast, during open mitosis the nucleolus is disassembled then reassembled. *Aspergillus nidulans* nuclei undergoes partially open mitosis, which is an evolutionary intermediate between open and closed mitosis. We therefore determined how *A. nidulans* nucleoli are segregated during mitosis. Unlike *Saccharomyces cerevisiae*, few *A. nidulans* nucleolar proteins segregate with DNA. Instead we have defined two patterns by which different nucleolar proteins segregate during mitosis: (1) Dispersal into the cytoplasm at the onset of mitosis but with some protein remaining bound to DNA, (2) A novel pattern in which nucleolar proteins remain in a nuclear remnant, distinct from daughter nuclei, before re-accumulating into daughter nucleoli during G1. Dual labeling of nucleolar proteins and nuclear envelope markers reveal that the nucleolar remnant is generated as a result of a double nuclear envelope fission event. This double fission occurs around a nucleolar protein mass during telophase. This mechanism generates two transport competent daughter nuclei and a very transient nucleolar remnant containing class 2 nucleolar proteins. This study indicates *A. nidulans* undergoes mitotic disassembly then reassembly of its nucleolus, as do higher eukaryotes, and that generation of daughter nuclei occurs via a double fission mechanism, not a single fission as occurs in yeasts. We suggest the novel mitotic nuclear remnant we have defined serves as a storage pool from which equal distribution of nucleolar proteins occur. It may also serve as a sink for unwanted cytoplasmic proteins or RNAs that gain access to nuclei during mitosis and may be a positional cue for the double fission.

36. **Genetic dissection of phosphate acquisition in *Neurospora crassa*.** Wayne K. Versaw, Yuan Jin, Lauren Baber and Kelly Guido. Dept of Biology, Texas A&M University, College Station, TX 77843

Depending on environmental conditions, *N. crassa* obtains phosphate via a constitutive, low-affinity uptake system or a phosphate-repressible, high-affinity uptake system. The high affinity system consists of two structurally unrelated transporters, PHO-4 and PHO-5. Homologous transporters with similar functional attributes have been well described in the yeast *Saccharomyces cerevisiae*. Low affinity phosphate transporters also have been described in yeast but based on mutant analyses, the single corresponding *N. crassa* homolog, PHO-6, does not appear to have a significant role in phosphate uptake. We describe a putative transporter, PHO-7, and evidence suggesting that PHO-7 constitutes the low-affinity phosphate transport system in *N. crassa*. PHO-7 represents a novel class of transport proteins that is conserved in filamentous fungi but is not represented in the yeasts.

37. **The *Aspergillus nidulans snxA1* and *nimA5* mutations interact to affect mitotic spindle structure.** Yulon Stewart, Ryan Day, Kirk Jackson, Michael Jackson, and Sarah Lea McGuire. Millsaps College, 1701 N. State St., Jackson, MS, 39210.

Both the *nimA* and *snxA* genes interact with *nimX*^{cdc2} to affect mitosis in *Aspergillus nidulans*. *nimA* affects the nuclear import of *nimX*^{cdc2}, while the *snxA1* mutation is a cold-sensitive suppressor of the *nimX2*^{cdc2} mutation. *snxA1* leads to abnormal nuclear morphology at 17ºC. To better understand effects of the *snxA1* mutation on cells and the relationship between *snxA* and *nimA*, we generated strains expressing GFP-*tubA* (alpha-tubulin) and various combinations of *snxA* and *nimA* mutations. At 17ºC *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 others were bifurcated or trifurcated and had fragmented, variably condensed nuclei along their lengths. Similar abnormal nuclei and spindle structures were observed when *snxA1/nimA1/*GFP-*tubA* cells and *snxA1/nimA5/*GFP-*tubA* cells were germinated at 32ºC and upshifted to 44ºC for 3 hours, suggesting that the effects of *snxA1* on *nimA* are not allele-specific. After 3 hours at 44ºC, 69% of *snxA1/nimA1/*GFP-*tubA* cells had abnormal nuclei, and 56% had abnormal spindles; similar results were obtained with *snxA1/nimA5/*GFP-*tubA* cells. Confocal microscopy of the abnormal spindles shows highly unusual spindle structures, which are more severe in cells carrying the *snxA1/nimA5* double mutant; *snxA1/nimA1* double mutant cells often have significantly shortened spindles. Efforts to clone the *snxA* gene are ongoing and should aid in the understanding of the interactions of the *snxA* and *nimA* genes in mitotic control. Supported by NIH R15GM55885 and NIH RR016476 from the MFGN INBRE program of the NCRR.

38. Withdrawn

39. **Cell Biology of Biotrophic Blast Invasion by the Rice Blast Fungus *Magnaporthe grisea*** Prasanna Knakanala[1], Kirk Czymmek[2], Barbara Valent[1]. [1]Kansas State University, Manhattan, KS [2]University of Delaware, Newark, DE

The filamentous ascomycete fungus, *Magnaporthe grisea* is a hemibiotroph that causes rice blast disease accounting for 60 million tons of grain loss annually. The blast fungus produces intracellular Invasive Hyphae (IH) to colonize the plant, but detailed cellular and molecular mechanisms by which this occurs were not studied. We applied live- cell imaging to characterize

spatial and temporal development of IH and plant responses inside successively-invaded rice cells. Early loading experiments with the endocytotic tracker, FM4-64, showed dynamic plant membranes around IH. These hyphae showed remarkable plasticity and recruited plant cell components. IH exhibited pseudohyphal growth and were sealed in plant membrane, termed the Extra-Invasive Hyphal Membrane (EIHM). The fungus spent up to 12 hours in the first cell, often tightly packing it with IH. IH that moved into neighboring cells were biotrophic, although they were initially thinner and grew more rapidly. IH in neighboring cells were wrapped in EIHM with distinct membrane caps at the hyphal tips. Time-lapse imaging showed IH scanning plant cell walls before crossing them, and transmission electron microscopy showed crossing occurring at pit fields. This and additional evidence strongly suggest that IH co-opt plasmodesmata for cell-to-cell movement. Our studies have revealed insights into a novel hemibiotrophic strategy employed by the blast fungus. Analysis of biotrophic blast invasion will significantly contribute to characterization of secreted fungal effectors that impact normal plant processes. To further understand the molecular basis of this infection strategy we have employed laser microdissection to identify the effectors involved in these processes.

40. **Identification of transcription factor regulated by OS-2 MAP kinase in *Neurospora crassa*.** Azusa Shiozawa[1], Shinpei Banno[2], Kazuhiro Yamashita[1], Setsuko Watanabe[1], Fumiyasu Fukumori[1], and Makoto Fujimura[1]. [1] Dept of Life Sciences, Toyo Univ, Gunma, Japan. [2]PRRC., Toyo Univ, Gunma, Japan.

Expression of the genes for glycerol synthesis (*gcy-1*, *gcy-3*, and *dak-1*), catalase (*ctt-1*), and gluconeogenic key enzymes (*fbp-1* and *pck-1*) are stimulated by osmotic stress and fludioxonil in OS-2 MAP kinase dependent manner in *Neurospora crassa*. To identify the transcriptional factor regulated by OS-2 MAP kinase, we isolated three disruptants for *atf-1* (CREB-type), *msn-1* (STRE-type), and *nap-1* (AP-1-type) genes, which are orthologs of *SKO1*, *MSN2/4* and *YAP1* of budding yeast, respectively. The *msn-1* disruptant stimulated aerial hyphae on agar medium and showed increased resistance to oxidative stress such as menadione and *t*-butyl hydroperoxide. In contrast, the *nap-1* disruptant was sensitive to oxidative stress. No gene disruption including *atf-1* affected sensitivity to osmotic stress and fludioxonil. However, fludioxonil-induced activation of the OS-2-dependent genes was almost thoroughly cancelled in the *atf-1* mutant but not in the *msn-1* and *nap-1* mutants. The *atf-1* mutant grew well on the agar medium, but no progeny with *atf-1* was obtained when the *atf-1* mutant was crossed with the wild-type strain. These data suggested that the transcription factor ATF-1 may acts downstream of OS-2 MAP kinase, and probably plays an important role in the viability of ascospore.

41. **Expression of the glucose-repressible *grg-1* gene is stimulated by osmotic stress and fludioxonil in OS-2 MAP kinase dependent manner**. Setsuko Watanabe[1] , Shinpei Banno[2] , Azusa Shiozawa[1] , Noriyuki Ochiai[3] , Makoto Kimura[3] , Makoto Fujimura[1]. [1]Dept of Life Sciences , Toyo University , Gunma , Japapan. [2]PRRC., Toyo University , Gunma , Japapan. [3]Env.Mol.Biol., RIKEN , Yokohama , Japan.

Two-component histidine pathway consisting of OS-1 (histidine kinase) and OS-2 (MAP kinase) plays an important role in osmotic regulation in *Neurospora crassa*. The cAMP-PKA pathway, comprised of MCB/HAH (regulatory subunit) and PKAC-1 (catalytic subunit), participates in the switching of conidiation and filamentation probably in response to carbon sources. The *hah;os-2* double mutant was more sensitive to osmotic stress than the *os-2* mutant. In contrast, the *pkac-1* mutation partially suppressed osmotic sensitivity of the *os-2* mutant, suggesting crosstalk between these pathways. Furthermore, we found that expression of the *grg-1* (glucose- repressible gene) gene was regulated under the OS pathway. Real-time PCR analysis demonstrated that transcription of the *grg-1* gene was stimulated by osmotic stress and fludioxonil in the wild-type strain but not in the *os-2* mutant. The GFP fluorescence intensity of the strain with the *Pgrg-1-sgfp* fused gene was significantly increased by osmotic stress and fludioxonil. There are several elements in the promoter region of the *grg-1* gene such as CRE (cAMP-Responsive Element) and STRE (Stress Responsive Element). Promoter analysis is currently being undertaken.

42. Withdrawn

43. **Analysis of DNA damage checkpoint genes in *Neurospora crassa***. Mitiyoshi Wakabayashi and Suuitu Tanaka. Saitama Univ. Regulation Biology, Saitama, Japan.

Checkpoint is an important mechanism for both DNA repair and chromosome maintenance. This mechanism has been studied in many organisms including yeast and human cells. In *Neurospora crassa*, various DNA repair genes have been studied in, but any studies concerning the DNA damage checkpoint had not been done. Recently, the clock gene *prd-4* in *Neurospora* was identified as a homologue of Human *Chk2*. It is meaning that a relationship between DNA damage checkpoint and circadian clock exists. But data about the role of *prd-4* toward both DNA damage checkpoint and DNA repair was not so much. So, we investigated three genes, that is one human *Chk1* homologue and two *Chk2* homologues. In general, checkpoint-deficient mutants show higher sensitivity against many mutagens than the wild type. In the *S.cerevisiae RAD53* (*Chk2* homologue) mutant is sensitive to some mutagens containing camptothecin (CPT), which causes replication fork collapse, and hydroxy urea (HU), that induces stall of replication fork. The *Neurospora Chk1* (*ncchk1*) disruptant indicated sensitivity to both CPT and HU. And two *Neurospora Chk2* (*nccds1* and *prd-4*) disruptants indicated sensitivity to CPT but not to HU. Moreover, we tried to make the relationship between these genes and other checkpoint genes clear. Surprisingly, CPT sensitivity of *mus- 9* (*Neurospora ATR*) was

suppressed by *nccds1* mutation. In yeast and human, CHK2 activation is dependent on ATR or ATM kinase activity, but such a genetic relationship observed in *Neurospora* had not be reported. These results indicate that *Neurospora crassa* has a unique mechanism in regulation of the DNA damage checkpoint.

44. **A single amino acid determines GAP-specificity of duplicated Rho-GTPases in *Ashbya gossypii*.** Michael Köhli[1], Sabrina Buck[2] and Hans-Peter Schmitz[1,2]. [1]Applied Microbiology, Biozentrum Universität Basel, Klingelbergstr. 50-70, 4056 Basel, Switzerland [2]Department of Genetics, University of Osnabrück, Barbarastr. 11, 49076 Osnabrück, Germany, hans-peter.schmitz@biologie.uni-osnabrueck.de, Tel.: +49 541 969 2289, Fax: +49 541 969 2293

While it is a known fact that gene duplication is a driving force of evolution, the resulting functional diversification is often unknown. We investigated the changes in function of the tandem duplicated homologs *Ag*Rho1a and *Ag*Rho1b of the *Saccharomyces cerevisiae RHO1* gene in the filamentous fungus *Ashbya gossypii*. We could show that the main differences between the two duplicated copies are due to a single amino acid mutation in the switch I region of one of the two *AgRHO1* genes. This exchange alters the specificity for the two Rho1-GAPs *Ag*Sac7 and *Ag*Lrg1 as shown by mutant analysis, interaction studies and GAP-assays with purified proteins. Our results explicitly show an example how gene duplication and single amino acid changes can rewire signal transduction networks during evolution.

45. **Role of Microtubule Organizing Centers for the Distribution, Oscillation and Division of Nuclei in the Filamentous Fungus *Ashbya gossypii.*** Claudia Birrer, Sandrine Grava, Tineke van den Hoorn, Dominic Hoepfner and Peter Philippsen, Molecular Microbiology, Biozentrum, University of Basel, Klingelbergstrasse 50/70, 4056 Basel, Switzerland, Claudia.lang@stud.unibas.ch

Nuclear positioning is important for normal growth and development of all eukaryotes including filamentous fungi. Among those, *Ashbya gossypii* is a particularly attractive organism to study nuclear distribution in multi-nucleated hyphae. Its 9 MB genome is completely sequenced and the annotation revealed a high degree of synteny to *Saccharomyces cerevisiae*. Using GFP-labled histone H4 nuclear dynamics including oscillation, bypassing and division have already been studied earlier (Alberti-Segui et al. 2001, Gladfelter et al. 2006). These processes are most likely co-regulated by the activities of microtubule organizing centers (MTOCs) embedded in the nuclear membrane and the dynamics of an elaborated microtubule (MT) network. We monitored MTOCs in *A. gossypii* hyphae carrying YFP-labled Tub4, the *A. gossypii* homolog of gamma-tubulin. Furthermore we studied the locations of re-emerging MTs after nocodazole treatment. Our data exclude hyphal tips or septa as locations for MTOCs and confirm *A. gossypii* nuclear-localized MTOCs as places of nucleating MTs. By *in vivo* labeling of alpha-tubulin with GFP and immuno-staining we could show that MTOCs nucleate two kinds of MTs: long ones interconnecting nuclei and short ones contacting the cell cortex. We also analyzed the function of AgSpc72, another component of *A. gossypii* MTOCs. Its homolog in *S. cerevisiae* is part of the outer, cytoplasmic layer of MTOCs. Yeast cells lacking this component only generate very short MTs. We show, that *AgSPC72*-deleted hyphae still form long cytoplasmic MTs, short MTs were not observed. Nuclear migration is substantially disturbed: nuclei are clustered distally from the tip, and nuclear oscillations are abolished. Moreover, the fraction of mitotic nuclei is twofold increased compared to wild type. To gain further insight into the controlled distribution and movement of nuclei we have started to investigate the role of other components on nuclear dynamics in *A. gossypii*.

46. **Mitochondrial transport in *Neurospora crassa* by the motor proteins NKIN2 and NKIN3**. Daniela Justa[1], Jolante Reth[2], Guenther Woehlke[2] and Stephan Seiler[1]. [1] Institute of Microbiology and Genetics, Goettingen, Germany [2] Institute for Cell Biology, Munich, Germany

The most widely used mechanism for intracellular transport involves molecular motor proteins that carry cargo directionally along cytoskeletal tracks (myosins along actin filaments and kinesins and dyneins along microtubules). Several proteins that interact with motor proteins have been identified, but our knowledge about how the motor-cargo interaction takes place and how it is regulated is still limited. Recently, it has been shown that two kinesins of the kin3 family (named KIN2 and KIN3) are associated with mitochondria *in vitro*. To further characterize this interaction, we have generated mutants in both kinesins. Phenotypic analysis suggests that KIN2 is the main motor responsible for mitochondrial motility in vegetative hyphae, but also that its loss can be compensated by KIN3. The inability of *kin-3* deletion strains to produce spores implicates an important function of this motor during sexual development. Currently, we are generating a *kin-2;kin-3* double mutant to analyze the partially overlapping functions of the two motors in more detail. Life imaging of GFP-tagged versions of KIN2 and KIN3 is being developed to study the dynamic localization of the motor proteins. To further dissect the interacting domains of the motors and the responsible mitochondrial receptors, we are generating myc-tagged constructs of both kinesins to purify associated components.

47. **Polar tip extension is coordinated by POD6 and COT1 in a motor protein-dependent manner in *Neurospora crassa***. Sabine Maerz[1], Nico Vogt[1], Carmit Ziv[2], Oded Yarden[2] and Stephan Seiler[1]. [1]University of Goettingen, Germany [2]The Hebrew University of Jerusalem, Israel

Members of the Ste20 and NDR protein kinase families are important for cell differentiation and morphogenesis in various organisms. We characterized POD6 (NCU02537.2), a member of the GCK family of Ste20 kinases that is essential for hyphal tip extension and coordinated branch formation in *N. crassa. pod-6* and the NDR kinase mutant *cot-1* exhibit indistinguishable growth defects, characterized by cessation of cell elongation, hyperbranching, and altered cell-wall composition. We suggest that POD6 and COT1 act in the same genetic pathway, based on the fact that both *pod-6* and *cot-1* can be suppressed by 1) environmental stresses, 2) altering PKA activity, and 3) common extragenic suppressors. Unlinked noncomplementation of *cot-1/pod-6* alleles indicates a potential physical interaction between the two kinases, which is supported by coimmunoprecipitation, yeast two hybrid studies, partial colocalization of both proteins in wild-type cells, and their common mislocalization in dynein/kinesin mutants. We conclude that POD6 acts together with COT1 and is essential for polar cell extension in a motor protein-dependent manner in *N. crassa*. We are currently developing enzyme assays for both kinases and are in the process of establishing an interaction network on the basis of yeast two hybrid studies and co-affinity purifications.

48. **Spitzenkörper localization and intracellular traffic of GFP-labeled class I and class VI chitin synthases in living hyphae of *Neurospora crassa***. M. Riquelme[1], S. Bartnicki-Garcia[1], J.M. González-Prieto[2], E. Sánchez- León[1], J. A. Verdín-Ramos[1], A. Beltrán-Aguilar[1], and M. Freitag[3]. [1]Department of Microbiology. Center for Scientific Research and Higher Education of Ensenada (CICESE). Km 107 Ctra. Tijuana-Ensenada. 22860 Ensenada, Baja California, México; [2]Center for Genomic Biotechnology. Blvd. del Maestro s/n. 88710 Cd. Reynosa, Tamaulipas, México; [3]Department of Biochemistry and Biophysics ALS 201. Oregon State University, Corvallis, OR 97331-7305, USA.

Chitin synthases (CHS) are essential enzymes involved in cell wall synthesis in fungi and arthropods. Little is known about their intracellular movement in growing fungal hyphae. Here, we present data on the localization of two selected CHS, CHS-3 and CHS-6, from *Neurospora crassa*. Both CHS-3 and CHS-6 were labeled at their carboxyl terminus with green fluorescent protein (GFP). We used high-resolution laser scanning confocal microscopy (LSCM) to analyze the localization and trafficking of CHS-3 and CHS-6 in growing hyphae. CHS-3-GFP and CHS-6-GFP showed similar distribution patterns along hyphae. In distal regions (beyond 45 µm from the tip), CHS-GFP was found mainly in a highly stained network of large endomembranous compartments and in nearby developing septa. At the subapex, the fluorescence was observed in numerous vesicles or groups of vesicles that moved predominantly forward until reaching the most proximal subapical region (15-20 µm from the tip). At the tip, the fluorescence congregated into a conspicuous single body corresponding to the location of the Spitzenkörper (Spk). Co-labeling of the Spk with the fluorescent marker FM4-64, showed CHS-GFP localized in the inner core of the Spk, the same region in which microvesicles were previously detected by transmission electron microscopy. Analysis of fluorescence recovery after photobleaching (FRAP) suggested that fluorescence at the Spk stems from the immediately surroundings vesicles. There was no co-localization of CHS-GFP fluorescence with stained secretory compartments fluorescence suggesting that CHS-3 and CHS-6 are not transported via the classical ER to Golgi to cell surface secretory pathway.

49. **The *Phycomyces blakesleeanus madB* gene is a member of the White Collar 2 family**. Catalina Sanz[1] and Arturo P. Eslava[1]. [1]Área de Genetica, Centro Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca, Spain catsof@usal.es, eslava@usal.es

Phycomyces blakesleeanus is a filamentous fungus that has been used as a model in several studies of environmental signals as light, chemicals, wind, gravity and adjacent objects. The *P. blakesleeanus* genome sequence has led to the identification of three putative homologous genes belonging to the White Collar 1 family of blue- light photoreceptors and four putative homologous genes (*wctA*, *wctB*, *wctC* and *wctD*) from the White Collar 2 family. In a recent work, we have found that mutations in one of those White Collar 1 homologous genes are responsible for the blind phenotype observed in the *madA* strains, and as such, this gene has been named *madA* (Idnurm et al., 2006). In the present work we have analyzed the White Collar 2 family (*wct* genes) in several *P. blakesleeanus* strains that exhibit an abnormal sensitivity to light (from *madA* to *madJ*) in order to detect possible mutations. All the analyzed *madB* strains were affected in the *wctA* gene and present the same G+906A mutation, so it seems likely that all the strains are from the same origin. No other mutations in the White Collar 2 gene family were found among the rest of the *mad* strains. The G to A change presumably prevents the splicing of the first intron of the gene. We have amplified the cDNA copy of the *wctA* gene in the *madB* mutants by PCR and have observed that these cDNAs are partially unspliced. The cDNAs from the *madB* strains C111, C112 and C109 have been cloned and sequenced to ensure that the mutation does indeed prevent the splicing of the first intron, and to show that there are no secondary splicing events. We have also analyzed some genetic crosses of the wild type strain with some *madB* strains and have looked for recombinants to confirm whether the wild type recombinants do have the wild type copy of the gene, while the *madB* recombinants have the G to A change. As all the *madB* strains show impaired light-sensing this phenotype seems to be link to the mutation found, therefore we have decided to name the *wctA* gene *madB*.

50. **The role of the exocyst in growth and morphogenesis of *Neurospora crassa* hyphae.** A., Beltrán-Aguilar[1], S., Seiler[2], and M., Riquelme[1]. [1]Center for Scientific Research and higher Education of Ensenada (CICESE), Department of Microbiology. Km.

107 Ctra. Tijuana-Ensenada, 22860, Baja California, México. [2] Institut für Mikrobiologie und Genetik Abt. Molekulare Mikrobiologie und GenetikGeorg-August- Universität Göttingen Grisebachstr. 8D-37077 Göttingen, Germany.

Exocytosis is the last step of secretion and it consists on vesicle incorporation to the plasma membrane. A high fidelity protein-protein interaction (mediated by Sec proteins, SNAREs, GTPases and the exocyst) helps to provide specific recognition at the exocytosis sites. The secretory machinery involved in this process has not been fully described in filamentous fungi, but most of its components are evolutionary conserved among eukaryotes from yeast to mammals' neurons. The exocyst complex plays an important role in the vesicle-plasma membrane interactions previous to SNAREs assembly and it is compossed of eight proteins (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84). Using a *Neurospora crassa sec5* mutant strain we have analyzed the importance of this component for Spitzenkörper formation, polarized growth and hyphal morphology. At low magnification *N. crassa sec5* showed a button like colony morphology, with hyperbranched and distorted hyphae. The mutant growth rate was 2.03% that of the wild type strain. At higher magnification the intracellular organization was severely affected. One of the key differences between the wild type and the mutant was the lack of a Spitzenkörper in the latter under high-resolution phase-contrast video- microscopy. A vector containing a *sec5::sgfp* the fusion was introduced into a *N. crassa* wild type strain and the localization and traffic of SEC5-GFP was analyzed by laser scanning confocal microscopy. Fluorescence did not accumulate at the tip, but was observed in a punctuate pattern all along the hyphae. This study establishes the basis for future analyses directed to elucidate the role of the exocyst in the apical growth of filamentous fungi.

51. Putative mannose transporters complement a branching/septation defect in *Aspergillus nidulans*. Loretta Jackson-Hayes, Lauren Fay, Terry W. Hill and Darlene M. Loprete. Departments of Biology and Chemistry, Rhodes College, Memphis, TN 38112. jacksonhayesl@rhodes.edu

In order to identify novel genes affecting cell wall integrity, we have generated mutant strains of the filamentous fungus *Aspergillus nidulans*, which show hypersensitivity to the chitin synthase inhibitor Calcofluor White (CFW). The phenotype of one of these strains (R205) also shows morphological abnormalities related to branching and septation. We have cloned two DNA fragments from an *A. nidulans* genomic DNA library which improve resistance to CFW and restore a more normal phenotype. One fragment is gene AN8848.3, "MT1", which shows homology to GDP-mannose transporters. The second fragment is gene AN9298.3, "MT2", which is a similar but distinct gene also homologous to GDP-mannose transporters. When separately cloned, the putative GDP-mannose transporters restore normal phenotype including full restoration of subapical hyphal compartment length and branch density in the mutant. Sequencing reveals a genetic lesion in Exon 5 of MT1 which causes an alanine to proline substitution and no mutation in MT2 in mutant strain R205. Cloned R205 MT1 containing the Exon 5 mutation does not complement the R205 phenotype. Attempts to produce null mutants of MT1 did not produce viable transformants, suggesting that AN8848.3 is an essential gene. MT2 null mutants grow normally under normal growth conditions and show wild type CFW resistance.

52. **Constitutive PKA activity prevents growth arrest of *Aspergillus fumigatus* conidia.** Kevin Fuller, Wei Zhao, Lauren Fox, Judith Rhodes. University of Cincinnati, Cincinnati, OH

We have previously generated a mutant of *A. fumigatus* deficient in the regulatory subunit of PKA and described that conidia are hypersensitive to various forms of oxidative stress. We also noted that the mutant conidia are larger than those of wt in distilled water. Both swelling and increased sensitivity to oxidative stress accompany metabolic activation after exposure of resting conidia to a germinant. Accordingly, we wanted to determine if the increase in conidial size of the *pkaR* deletion mutant was due to an unexpected initiation of germination in water. To determine the metabolic state of the conidia, we evaluated mitochondrial activity with Mitotracker Orange. Whereas it required 4 hours in a rich medium for the wt and complemented strains to stain positively, the *delta pkaR* conidia were positive at earlier time points, even in the absence of a germinant. Furthermore, the mutant conidia were killed more readily by hydrogen peroxide when compared to the resting conidia of the other isogenic strains. However, after the 4 hour time point, all members of the isogenic set showed equivalent killing by the treatment. We, therefore, conclude that the hypersensitivity to hydrogen peroxide seen with the conidia of the *pkaR* deletion mutant is due to failure of resting conidia to maintain growth arrest.

53. **Ras signaling in the human pathogen *Cryptococcus neoformans.*** Connie B. Nichols and J. Andrew Alspaugh. Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA. connie.nichols@duke.edu

Cryptococcus neoformans is an opportunistic human fungal pathogen that is the causative agent of cryptococcosis, a life-threatening infection. The ability to grow at high temperature is an important requirement of *C. neoformans* to establish disease. Previously we found that Ras1 was required for growth at high temperature and for sexual differentiation. We have now identified the downstream components of Ras1 that mediate the response to high temperature, including Cdc24, Cdc42, and Ste20. In the fission yeast *Schizosaccharomyces pombe*, Ras mediates morphology and sexual differentiation using different upstream activators and downstream effectors. To understand how Ras1 mediates both high temperature growth and sexual differentiation in *C. neoformans* we have generated a series of *ras1* mutant strains including dominant negative, dominant active,

farnesylation-defective, and palmitoylation-defective *ras1* mutant strains. In addition we have used genetic epistasis experiments to characterize the role of the Cdc24-Cdc42-Ste20 branch of Ras1 signaling in sexual differentiation in *C. neoformans*.

54. **Two-component signaling system for osmotic regulation and fungicide sensitivity in *Cochliobolus heterostrophus*.** Chihiro Tanaka, Kosuke Izumitsu, and Akira Yoshimi* Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, JAPAN. *Present address: New Industry Creation Hatchery Center (NICHe), Tohoku University, Aoba, Sendai 980-8579, JAPAN. E-mail: chihiro@remach.kais.kyoto-u.ac.jp

We have studied the mode of action of dicarboximide/phenylpyrrole fungicides, using resistant mutants of a maize pathogen *Cochliobolus heterostrophus*. In this fungus, the resistant mutants always showed hyperosmotic sensitivity, as reported in *N. crassa*. And *dic1* encoding a histidine kinase (HK) was characterized as a responsible gene for fungicide resistance and osmotic adaptation. We also elucidated that exposure to the fungicides led to Dic1p mediated improper activation of Hog1-MAPK (BmHog1p). HK and MAPK are core components in two- component signaling and MAPK signaling systems, respectively. These results suggested that both signaling systems were involved in the mode of the action of the fungicides and high-osmorality adaptation in this fungus. To investigate further, we isolated mutants of *ChSln1* (HK, whose orthologue is a sole HK in yeast and involved in its osmo-adaptation), *ChSsk1* (response regulator: RR) and *ChSkn7* (another RR) and compared their phenotypes with a wild- type, *dic1* and *Bmhog1*. *ChSln1* disruptants showed no apparent phenotypic changes with respect to osmo-sensitivity. However, both of *Chssk1* and *Chskn7* mutants showed moderate resistance to the fungicides, and some sensitivity to high osmorality. The phenotype comparisons and phosphorylation analyses of Hog1- MAPK in these mutants revealed that *C. heterostrophus* has two streams of two-component signaling pathways for the osmotic adaptation and fungicide sensitivity: one is Dic1p-ChSsk1p driving Hog1-MAPK cascade, another is Dic1p-ChSkn7p, and both streams were controlled by Dic1p.

55. **Live cell imaging of microtubule dynamics in *Neurospora crassa* using total internal reflection fluorescence microscopy**. Maho Uchida[1], Rosa R. Mouriño-Pérez[2], Michael Freitag[3] and Robert W. Roberson[1] [1]School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA [2]Departamento de Microbiología. Centro de Investigación Científica y Educación Superior de Ensenada. Ensenada, B. C. Mexico [3]Department of Biochemistry and Biophysics, Oregon State University, Eugene, OR 97331-7305, USA

Total internal reflection fluorescence (TIRF) microscopy is an optical technique that uses evanescent waves to excite fluorophores within 200 nm of the specimen and coverslip interface. This effectively eliminates background fluorescence from other regions of the cell and improves spatial resolution by increasing the signal-to-noise ratio. Furthermore, living cells can be viewed over extended time periods due to low phototoxic effects. Here, we used TIRF microscopy to study cortical microtubule (MT) dynamics in living primary hyphae of *Neurospora crassa* expressing β-tubulin- GFP. Detection of plus-end MT dynamics was much improved with this approach compared to confocal and widefield fluorescence microscopy methods. Surprisingly, MTs in *N. crassa* polymerize twice as fast as those reported in *Aspergillus nidulans*, while MT depolymerization rates in both species were similar. During this study, we observed events of MT fragmentation and fragment motility. These behaviors were compared to those observed in MT-motor mutant strains in order to address the role(s) played by dynein/dynactin and kinesin in MT behavior.

56. **Micro and nano morphology of Aspergillus species in response to carbon starvation.** Mark R. Marten, Judith Kadarusman, Liming Zhao, Youghyun Kim, Bill Moss Zebulon Jones and David Schaefer[2]. UMBC, Chemical & Biochemical Engineering, Baltimore, MD 21784, [2]Towson Univ., Physics Dept, Towson, MD 21252

We are using Aspergillus species as models to characterize fungal morphological response to carbon starvation. To study micro-morphology we use a parallel-plate flow chamber, with a narrow (50 micron) gap, forcing two dimensional fungal growth. This chamber is perfused with oxygenated growth medium, and mounted on a microscope stage, allowing us to follow morphological development of individual mycelial as a function of time and growth environment. In response to carbon deprivation (i.e., step change, glucose$^+$ to glucose$^-$), *A. oryaze* enters a lag phase where extention ceases, vacuolation increases, and duration depends on the initial size of the mycelium. When complete, regrowth begins at a relatively constant rate independent of mycelial size. When this behavior is compared to that during rapamycin-induced autophagy we find quantitative differences implying autophagy may comprise only a portion of a more complicated starvation stress response. To study nano-morphology we use an atomic force microscope (AFM). We have developed the ability to use the AFM for testing material properties of fungal cell walls as a function of axial position. We will discuss development of this technique and how we are using it to study changes in *A. nidulans* morphology during carbon deprivation.

57. Withdrawn

58. **A functional analysis of the DopA protein of *A. nidulans* and *A. fumigatus***. Holger Hannemann and Bruce Miller. University of Idaho, MMBB. Moscow, Idaho

Aspergillus nidulans (*An*) is a widely used model organism whereas *A. fumigatus* (*Af*) is an airborne pathogen, causing mostly fatal invasive mycoses in immunocompromised patients. Polarized growth is one of the key factors of fungal pathogenesis. DopA, whose homologs are found in fungi, worms and chordata, is a protein involved in polarization of cells. An *An* strain expressing a temperature sensitive DopA protein (DopAts) displays aberrant cellular morphology of all cell types, asynchronous cell pattern formation and loss of both asexual and sexual reproductive cycles at the restrictive temperature. Successive shortening of the *An* and *Af dopA* open reading frames demonstrated that the leucine zippers, located at the C-terminal end of the proteins, serve different functions in these two organisms. DopA-GFP fusions in both *An* and *Af* revealed that the proteins are localized to the Golgi and the endosomal membrane networks in both species. Genetic interactions between *An dopA1*ts and *An rasA* suggest that DopA modulates signaling through a RasA-mediated signal transduction pathway that controls cell polarity and morphogenesis. Additionally, the N-terminal dopey domain of *An* DopA interacts physically with BemA, the *S. cerevisiae* (*Sc*) Bem3 homolog. Therefore, the interactions between *An* RasA and *An* DopA, *An* BemA and *An* DopA and signaling interactions between *Sc* Ras and the *Sc* Cdc42 regulatory module suggests that the DopA/Dop1 protein plays a central role in integrating signal transduction and mechanisms regulating cell polarity in the fungi.

59. **The *Aspergillus nidulans snoA* inhibitor of cell division associates with the BRDF checkpoint domain of *nimO*^{Dbf4}.** Steve James, James Barra, Megan Campbell, and Matthew Denholtz. Biology Department, Gettysburg College, Gettysburg, PA. sjames@gettysburg.edu

In *Aspergillus nidulans*, *nimO*^{Dbf4} and *cdc7* encode regulatory and catalytic subunits of the conserved DBF4-dependent kinase (DDK). DDK initiates DNA synthesis by phosphorylating the replicative DNA helicase to trigger DNA unwinding at origins of replication. In addition, DBF4 plays an important role in the DNA damage response. This role is mediated by an N-terminal BRDF motif (*BR*CT and *D*B*F*4 similarity domain), as revealed by mutations in yeast homologs that confer enhanced sensitivity to DNA damage agents and failure to restrain DNA synthesis during genotoxic stress. In *Saccharomyces cerevisiae*, *RAD53/CHK2* kinase is the only checkpoint mediator known to associate directly with the *DBF4* BRDF motif. We identified a novel inhibitor of *nimO*^{Dbf4} called *snoA* (suppressor-of-nim*O*). Loss of *snoA* rescues *nimO*18 ts-lethality and hypomorphic *nimO*+ expression. Conversely, *snoA* overexpression confers a dose-dependent, lethal interphase cell cycle arrest in *nimO*18 cells. Here we report a novel interaction between the *nimO* BRDF motif and *snoA*. Using yeast two-hybrid analysis, we demonstrate that a short (~100 amino acid) serine- and proline-rich region in the *snoA* C-terminus can associate with the *nimO* BRDF. This novel discovery suggests that *snoA* may act to regulate normal DNA synthesis or to exert S phase checkpoint control by direct association with the *A. nidulans* DBF4-dependent kinase. (Supported by NSF-RUI #01-14446 to SJ)

60. **Where is calcium in the cell and how does it get there?** Barry Bowman, Stephen Abreu, Marija Draskovic and Emma Jean Bowman. Department of MCD Biology, University of California, Santa Cruz, CA 95060 email:bowman@biology.ucsc.edu

Calcium gradients are hypothesized to have a central role in polar growth. However, little is known about the distribution of calcium within fungal cells or the proteins that transport it. By fractionating cells we have found that mitochondria, vacuoles, and unidentified light organelles contain roughly equal amounts of calcium. The *Neurospora crassa* genome contains at least four P-type ATPases that appear to pump calcium - *nca-1*, *nca-2*, *nca-3*, and *pmr-1*. In addition seven genes encode transport proteins that may exchange calcium for H^+ or Na^+, *cax* being the best characterized. We have characterized nine mutant strains that lack a calcium transporter and have fused several transporters to GFP. The types of transporters and the distribution of calcium are more complex in *N. crassa* than in *S. cerevisiae*, the best studied system. Disruption of the *cax* gene causes the complete loss of calcium from the vacuoles, but does not affect other compartments in *N. crassa*. Yeasts lack a homolog of the endoplasmic reticulum Ca-ATPase of animal cells (the SERCA ATPase), but *N. crassa* has a homolog, *nca-1*. Interestingly, nca-1::GFP localizes largely to the nuclear envelope, suggesting the nuclear envelope forms a major part of the endoplasmic reticulum in fungi. Two of the mutant strains, *pmr-1* and one of the homologs of *cax*, are significantly altered in hyphal morphology. Do they help to generate calcium gradients at the hyphal tip?

61. **Withdrawn**

62. Withdrawn

63. **Two-component response regulators, ChSsk1p and ChSkn7p, additively regulate high-osmolarity adaptation and fungicide sensitivity in *Cochliobolus heterostrophus*.** Kosuke Izumitsu, Akira Yoshimi* and Chihiro Tanaka. Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, JAPAN. *Present adress: New Industry Creation Hatchery Center (NICHe), Tohoku University, Aoba, Sendai 980-8579, JAPAN.

Filamentous fungi possess many histidine kinases and two conserved response regulators, Ssk1p and Skn7p, in their two-component signaling systems. We have characterized the response regulator genes *ChSsk1* and *ChSkn7* in the Southern corn leaf bright fungus *Cochliobolus heterostrophus*. Both *Chssk1*- and *Chskn7*- disrupted mutants showed little sensitivity to high-osmolarity stress and moderate resistance to the iprodione/fludioxonil fungicides. The phosphorylation of Hog1-type MAPK BmHog1p induced by high-osmolarity stress and fungicide treatments was only regulated by ChSsk1p, indicating that ChSkn7p has roles in high-osmolarity adaptation and fungicide sensitivity that are independent from the activation of BmHog1p. The *ChSsk1*/*ChSkn7* double mutants clearly showed higher sensitivity to osmolar stress and higher resistance to fungicides than the single mutants. The dose responses of the double mutants fit well with those of the group III histidine kinase-deficient strain. These results suggest that in filamentous ascomycetes, the Ssk1-type and Skn7-type response regulators control high-osmolarity adaptation and fungicide sensitivity additively with differential mechanisms under the regulation of the group III histidine kinase.

64. **The role of cAMP-receptor-like genes in *Neurospora crassa* development.** Svetlana Krystofova and Katherine Borkovich, Plant Pathology, University of California Riverside, Riverside, CA 92521, svetlana@ucr.edu

G-protein coupled receptors (GPCRs) are 7-transmembrane proteins that sense a variety of extracellular stimuli, such as light, odor, chemoattractants, peptides, neurotrasmitters, hormones and lipids. Putative GPCRs have been identified in the *Neurospora crassa* genome sequence. Based on phylogenetic analysis, three of these genes, *gpr-1*, *gpr-2* and *gpr-3*, belong to a cAMP-receptor-like gene family that includes *Arabidopsis* GCR1, *Dictyostelium* crlA-crlC, *Cryptococcus neoformans gpr4* and *Aspergillus nidulans* GprH. Deletion of *gpr-1* leads to very specific defects during sexual development (lack of ostioles), while asexual development is not affected. The absence of ostioles in mature perithecia negatively contributes to the ability to release ascospores by an ejection mechanism. Moreover, perithecia are frequently ruptured leading to the release of inner material. The functions of *gpr-2* and *gpr-3* remain unknown. The expression profiles indicate that these genes are expressed in early developmental stages. Deletion mutants have been isolated and phenotypically analyzed. The possibility of redundancy between *gpr-1, gpr-2* and *gpr-3* has been investigated. Both genetic and biochemical approaches have been used to identify possible downstream effectors of *N. crassa* cAMP-receptor-like proteins.

65. **Role of cytoplasmic bulk flow and microtubule-related motor proteins in nuclear displacement in *Neurospora crassa* mature hyphae.** Silvia L. Ramos-Garcia[1], Rosa R. Mouriño-Pérez[1], Michael Freitag[2], Salomon Bartnicki-Garcia[1]. [1]Departamento de Microbiologia. Centro de Investigación Científica y Educación Superior de Ensenada. Ensenada B. C. Mexico. rmourino@cicese.mx. [2] Department of Biochemistry and Biophysics, Center for Genome Research and Biocomputing. Oregon State University. Corvallis, USA.

Nuclear movement and localization are multifactorial events that have been intensely studied over the last two decades. Here we report the results of a study to evaluate nuclear dynamics in the subapical region of Neurospora hyphae. Nucleus movement may be controlled by many factors, for example, cytoplasmic bulk flow, the cytoskeleton (microtubules and actin microfilaments) and motor proteins (dynein/dynactin complex and conventional kinesin). By confocal microscopy, the nuclear displacement of five strains of *Neurospora crassa* that express histone H 1 tagged with GFP was compared. We included a control strain, *ropy-1, ropy-3, nkin* and a *ropy-1; nkin* double mutant. Additionally, we treated the H1-GFP control strain with benomyl to inhibit microtubule formation and with cytochalasin A to inhibit actin microfilaments. Anterograde and retrograde movement of nuclei were observed in all strains, except the double mutant. The displacement rate of nuclei was highly correlated to hyphal elongation rate. The exception was the *nkin* mutant and cells treated with cytochalasin A. The shape of nuclei in growing hyphae was different: In the control, almost all nuclei were elongated, while in all other strains varying numbers of spherical nuclei were observed. The distance between the hyphal apex and the first nucleus varied between all strains and treatments: The *ropy-1* mutant showed the largest exclusion region and the cytochalasin A-treated cells completely lacked this nucleus exclusion zone. In conclusion, the movement and distribution of nuclei in mature hyphae appear determined by a combination of forces, with cytoplasmic bulk flow being a major determinant. Nevertheless, motor proteins bind nuclei to microtubules and actin microfilaments appear to act as a scaffolding to move all components in unison with the cytoplasm.

66. **Characterization of signaling genes *ncSCD2,ncSHK1* in *Neurospora crassa*.** Masashi Kawamura, Makoto Kataoka, Makoto Fujimura, and Akihiko Ichiishi. Faculty of Life Sciences, Toyo University, Gunma, Japan.

Schizosaccharomyces pombe has the SCD1 signaling pathway regulated by RAS1. This pathway is necessary for proper morphogenesis such as polarized growth or actin cytoskeleton, and pheromone response. In this pathway, SCD1 that is GDP/GTP exchange factor (GEF) for CDC42 (small GTPase) interacts with SCD2 (scaffolding protein). Then, GTP-bound CDC42 and SHK1 (p-21 activated kinase) also interact with SCD2. This complex transfers phosphate signals from up stream to down stream. Therefore, SCD2 and SHK1 function in the same pathway, both proteins are important factors mediate this pathway. *Neurospora crassa* also has the homologous of fission yeast SCD2 and SHK1, so we each named these genes *ncSCD2* and *ncSHK1*, respectively. Then, we constructed strains defective in *ncSCD2* and *ncSHK1* to characterize and analyze these genes function. Both null mutants grew more slowly than wild type strain, and displayed morphological defects that appear to have abnormal branching pattern and frequently septations. Moreover, both null mutants couldn't fertilize. But there were some differences in

phenotype between *ncSCD2* mutant and *ncSHK1* mutant. *SHK1* mutant resisted osmotic stress more strongly than *ncSCD2* mutant. This data suggests that *N.crassa* also has the SCD1 signaling pathway, and that *ncSCD2* and *ncSHK1* function resemble to fission yeast *scd2* or *shk1*. In addition, the fact that *SHK1* mutant had resisted high osmotic stress implied possibility that the SCD1 signaling pathway link to the HOG (High-osmolarity glycerol) pathway in *N.crassa*.

67. **Spatially segregated SNARE protein interactions in filamentous fungus.** Mari Valkonen[1], Markku Saloheimo[1], Merja Penttilä[1], Nick D. Read[2], and Rory R. Duncan[3], [1]VTT Biotechnology, P.O. Box 1000, FIN-02044 VTT, Finland. [2]Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3JH, UK. [3]Membrane Biology Group, University of Edinburgh, George Square, EH8 9XD, UK

The machinery for trafficking proteins through the secretory pathway is well conserved in eukaryotes, but remains poorly characterized in filamentous fungi. We describe the isolation of the *snc1* and *sso1* genes encoding exocytic SNARE proteins from *Trichoderma reesei*. The encoded SNCI protein can complement Snc protein depletion in *S. cerevisiae* whilst the *T. reesei* SSOI protein was unable to complement depletion of its yeast homologues. The localization and interactions of the *T. reesei* SNARE proteins were studied with advanced fluorescence imaging methods using fluorescent fusions of the SNARE proteins. The SSOI and SNCI proteins co-localized in sterol-independent clusters on the plasma membrane in sub-apical but not apical hyphal regions. The v-SNARE SNCI localized to the apical vesicle cluster within the Spitzenkörper of the growing hyphal tips when expressed under the *T. reesei cbh1* promoter. Using fluorescence lifetime imaging microscopy (FLIM) and fluorescence energy transfer (FRET) analysis, we quantified the interactions between these proteins with high spatial resolution in living cells. Our data showed that the site of SNARE complex formation between these proteins is on the plasma membrane of non- growing hyphae in old sub-peripheral regions of the colony, but that there is no interaction between the proteins in growing hyphal tips at the colony margin.

68. **A RasGAP protein involved in polarity establishment and maintenance in *Aspergillus nidulans.*** Laura Harispe[1,2,3], Lisette Gorfinkiel[2], Cecilia Portela[2], Miguel A. Peñalva[3], and Claudio Scazzocchio[1]. [1]IGM, Univ. Paris-Sud XI. Bat 409 Centre d´Orsay. 91405 Orsay(France). [2]F. Ciencias, Univ. de la República. Iguá 4525. 11400 Montevideo(Uruguay) [3]CIB, CSIC, Ramiro de Maeztu 9. 28040 Madrid(Spain)

Filamentous fungi represent an extreme example of polarised growth[(a)]. We report on the identification and characterisation of GapA, an *A. nidulans* RasGAP involved in polarity establishment and maintenance. GapA was identified after serendipitously isolating a partial loss-of-function mutation, designated *gapA1*, in a genetic screen. Phenotypic characteristics resulting from *gapA* deletion include compact colony morphology, a marked delay in polarity establishment during conidial germination, impairment of polarised hyphal extension, a conspicuous developmental defect typically manifested by the absence of one layer of sterigmata in the conidiophore and a defect in the otherwise polarised distribution of the actin cytoskeleton. GapA-GFP protein fusion expressed from a gene replacement allel appears localises to hyphal tips and septa. This localisation suggests that a Ras protein(s), whose activity is antagonised by GapA plays a role in the regulation of the actin cytoskeleton at the hyphal tip and that this abnormal regulation underlies the polarity phenotypes associated with *gapA* loss-of-function. [(a)]Momany M. (2002),Curr. Opinion in Microbiology, 5:580–585.

69. **Optical tweezer micromanipulation of living fungal cells.** Graham Wright[1], Jochen Arlt[2], Wilson Poon[2] & Nick Read[1]. [1]Institute of Cell Biology, University of Edinburgh, Edinburgh, UK. [2]School of Physics, University of Edinburgh, Edinburgh, UK graham@fungalcell.org

Optical tweezers facilitate the non-invasive micromanipulation of both inert and biological microscopic particles solely by using light. We are using optical tweezers in a range of cell biological applications. They have been used in various experimental live-cell studies to manipulate intracellular organelles, hyphal growth and branching, and whole cells. Optically trapped microbeads have been used for the localized delivery of chemicals and to mechanically stimulate cells. Finally, the effects of optical trapping on fungal cell viability have been assessed.

70. **Characterization of *radC*, the *Aspergillus nidulans* homolog of *RAD52*, a key gene for DNA repair by homologous recombination.** Michael Lynge Nielsen, Gaëlle Lettier, Jakob Blæsbjerg Nielsen and Uffe Hasbro Mortensen. Technical University of Denmark, BioCentrum-DTU

Repair of DNA double-strand breaks (DSBs) is crucial for maintaining genome integrity and failure to repair even a single DNA DSB is lethal as it causes loss of part of a chromosome during cell division. In the yeast, *Saccharomyces cerevisiae*, Rad52 plays a fundamental role in the repair of DSBs by homologous recombination (HR) and among genes involved in DNA DSB repair and HR deletion of *RAD52* produces the most dramatic phenotype. Hence, *S. cerevisiae* cells lacking Rad52 are impaired in DSB repair and all types of HR including targeted integration of transforming DNA. Orthologs of *RAD52* have been identified in other organisms, including *Schizosaccharomyces pombe*, *Aspergillus nidulans*, chicken, mouse, and human. However, in contrast to *S.*

cerevisiae, the absence of *RAD52* in higher eukaryotes does not result in a severe phenotype, due to the evolution of additional repair pathways and the role of Rad52 in DNA DSB repair and HR remains unclear. To address the role of Rad52 in higher eukaryotes, we have recently constructed a *radC* (homolog of *RAD52*) deletion mutant in *A. nidulans* and performed an initial characterization of the mutant strain. Unlike in *S. cerevisiae*, where Rad52 is essential for the repair of all types of DNA lesions that require HR, we show that in *A. nidulans* it is only required for the repair of a subset of these lesions. Hence, in an evolutionary perspective, HR repair in *A. nidulans* may represent an intermediate state between *S. cerevisiae* and human and *A. nidulans* may therefore be a useful model to further the understanding of HR in higher eukaryotes.

71. **A new cell type produced by conidia that is involved in sexual reproduction in *Neurospora crassa*.** Hsiao- Che Kuo, Gabriela Roca, Chris Jeffree and Nick D. Read. Institute of Cell Biology, University of Edinburgh, UK

We describe a new cell type, the conidial sex tube (CST), which is produced by macroconidia and arthroconidia of *Neurospora crassa*. It is morphologically and physiologically distinct from germ tubes and conidial anastomosis tubes (CATs), and under separate genetic control. CSTs are characterized by being long, thin (thinner than germ tubes), straight and unbranched. They contain few nuclei and do not avoid or grow towards each other. The CST surface texture and chemistry is different to that of germ tubes and CATs. CSTs require the presence of protoperithecia of opposite mating type to be induced (germ tube and CAT formation are inhibited under these coniditions). Trichogynes show strong chemotropisms towards CSTs but CSTs do not grow towards trichogynes. On making contact, trichogynes coil around CSTs. Trichogynes are not attracted to or coil around CSTs in mutants not producing sex pheromones when male only and trichogynes are not attracted to CSTs in mutants lacking sex pheromone receptor when female only. Nuclear division is inhibited in the presence of CSTs. CST formation is weakly conidium density dependent in contrast to CAT formation which is strongly conidium density dependent and germ tube formation which is conidium density independent.

72. **Organelle organization and behavior during CAT fusion and heterokaryon formation in *Neurospora crassa*.** Gabriela Roca[1], Michael B. Freitag[2] & Nick D. Read[1]. [1]Inst. of Cell Biology, University of Edinburgh, UK. [2]Dept. of Biochemistry and Biophysics, Oregon State University, USA

Conidia of *Neurospora crassa* form conidial anastomosis tubes (CATs) which are specialized hyphae that are distinct from germ tubes. CAT induction, homing and fusion were analyzed using strains expressing different GFP- or mRFP-fusion proteins, and compatible heterokaryons resulting from the fusion of these strains. Various organelle- selective dyes were also used. Cytoplasm passed through fused CATs prior to microtubules, and were followed by mitochondria and then nuclei. When heterokaryons were generated in which one of the parent homokaryons expressed either H1::GFP or H1::mRFP, it took > 3 h for nuclei in the unlabelled homokaryon to become labeled. Mitosis occured more slowly in ungerminated macroconidia (~ 1.5 h) than in germ tubes (~ 15 min). Mitosis was arrested during CAT homing and nuclei did not enter CATs. We also analysed heterokaryons resulting from CAT fusion between vegetatively incompatible strains differing in mating type. Nuclear immobilization and cell vacuolarization started 6 h after fusion but was not immediately followed by cell death. Heterokaryon incompatibility following CAT fusion is therefore delayed in contrast to the rapid cell death that follows incompatible fusions in mature colonies. This suggests that *N. crassa* possesses a mechanism to escape from vegetative incompatibility during the early stage of colony establishment.

73. **Intracelular Analysis of *Neurospora crassa* Germlings by Confocal Microscopy**. Castro-Longoria E., Araujo-Palomares C. and Riquelme M. ecastro@cicese.mx. Department of Microbiology, CICESE, Km. 107 C. Tij-Ensenada, 22860 Ensenada, B. C., México.

By laser scanning confocal microscopy and using the amphiphilic styril dye FM4-64 and *Neurospora crassa* wild type strain whose microtubules are labeled with GFP we have analyzed the intracellular changes prior to the appearance of a mature Spk. Observations began at the early stages of spore germination and were carried out until a conspicuous Spk could be observed. Before the Spk appearance, germ tubes (<150 µm) displayed a uniform distribution of organelles along the length of the cells. Once the germlings reached approximately 66.4 + 4.5 µm in length, a small accumulation of vesicles stained with FM4-64, but not detected by phase-contrast microscopy could be observed at the apex of the cell. When germlings reached more than 150 µm in length visible organelles such as mitochondria and nuclei experienced a displacement towards the subapical region of the cell and a small exclusion zone free of organelles formed at the apex. The position of this exclusion zone within the apex seemed to determine the germling growth direction, which was highly erratic. Few minutes after it first appeared, upon growth of the germling, the exclusion zone started to become occupied by an accumulation of material that gradually concentrated into a light gray body that we describe as an immature Spk, the accumulation of FM4-64 colocalized with the position the immature Spk. Our preliminary observations on microtubule organization during germ tube elongation showed that during the early stages of germination of *N. crassa*, cytoplasmic microtubules were less abundant, shorter and differently organized when compared to those previously reported in mature vegetative hyphae. At later stages, when a mature Spk was observed, cytoplasmic microtubules were more abundant, longer, and were distributed along the main longitudinal axis of growth of the germ tube. The formation of a mature Spk and a longitudinal and abundant distribution of microtubules coincided with the stabilization of the

growth direction of the germling, therefore suggesting that it is at this stage when the transition from germling to vegetative hypha occurs.

74. **Reversion Analysis of the Cytoplasmic Dynein Motor: a Trip Around the Ring.** Michael Plamann, David Madole, Robert Schnittker, and Elizabeth Wulff. School of Biological Sciences, University of Missouri-Kansas City

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 (MTBD) protrudes. Using a genetic assay, we have isolated over 50 DHC mutants of Neurospora that produce full-length proteins that are defective in function. We have identified DHC point mutations in nearly all domains within the dynein motor head. We have now isolated revertants for a subset of these DHC mutants and have identified the respective intragenic suppressor mutations in >100 revertants. Interestingly, we have found that two mutations within the MTBD are suppressed by mutations in various domains around the ring. These results suggest that the conformational states of the MTBD and motor head are tightly coupled. Most DHC mutations examined to date revert exclusively by intragenic suppression. However, 30 to 90% of the AAA#6 and C- terminal domain DHC revertants contain extragenic suppressors. These results suggest that AAA#6 and C-terminal domain mutations are readily bypassed by mutations in other genes.

75. **Endocytosis and polarized growth in *Aspergillus nidulans.*** Araújo-Bazán L., Peñalva M.A & Espeso E.A. CIB, C.S.I.C., Ramiro de Maeztu, 9 28040 Madrid (Spain).

In *Aspergillus nidulans* polarity maintenance during hyphal growth involves the polarised deposition of secretory vesicles at the extending tip. Cortical actin patches are present along the hyphal tube but strongly predominate in the apical region One third of the endocytic proteins directly regulate actin assembly and/or bind to actin. The unique *Abp1* and *SlaB* orthologue in *Aspergillus* were found using bioinformatics and being designated as *abpA* and *slaB*, respectively. After cDNA sequencing, we determined that AbpA shows strong similarity with Abp1p having one conserved cofilin domain and two, instead of one, SH3 domains. AbpA localises to highly motile and transient peripheral punctuate structures overlapping with actin patches. SlaB also localises to peripheral patches, which are markedly more abundant and cortical than those of AbpA but their motility is notably restricted as compared to AbpA patches. Similar subcellular localisation has been determined for actin and the amphiphysin orthologue AnRvs167. AbpA patches show random distribution during the isotropic growth phase preceding polarity establishment, but polarise as soon as a germtube primordium emerges from the swelled conidiospore. Thus, while endocytosis can occur along hyphae, the apical predominance and spatial organization of these endocytic machinery proteins as a slightly subapical ring strongly suggest that tight spatial coupling of secretion and compensatory endocytosis underlies hyphal growth.

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76. **Recombination hotspots flank the *Cryptococcus* mating-type locus: implications for the evolution of a fungal sex chromosome.** Yen-Ping Hsueh, Alexander Idnurm, and Joseph Heitman Department of Molecular Genetics and Microbiology, Duke University, Durham, North Carolina

Recombination increases dramatically during meiosis to promote genetic exchange and generate recombinant progeny. Interestingly, meiotic recombination is unevenly distributed throughout genomes and as a consequence genetic and physical map distances do not have a simple linear relationship. Recombination hotspots and coldspots have been described in many organisms and often reflect global features of chromosome structure. In particular, recombination frequencies are often distorted within or outside sex determining regions of the genome. Here we report that recombination is elevated adjacent to the mating-type locus (*MAT*) in the pathogenic basidiomycete *Cryptococcus neoformans*. Among fungi, *C. neoformans* has an unusually large *MAT* locus, and recombination is suppressed between the two >100 kb mating-type specific alleles. When genetic markers were introduced at defined physical distances from *MAT*, we found the meiotic recombination frequency to be ~20% between *MAT* and a flanking marker at 5, 10, 50 or 100 kb from the right border. As a result, the physical/genetic map ratio in the regions adjacent to *MAT* is distorted ~10- to 50- fold compared to the genome-wide average. Moreover, recombination frequently occurred on both sides of *MAT* and negative interference between crossovers was observed. Sequence analysis revealed a correlation between high G + C content and these hotspot regions. We hypothesize that the presence of recombinational activators may have driven several key events during the assembly and reshaping of the *MAT* locus, and may have played similar roles in the origins of both metabolic and biosynthetic gene clusters. Our findings suggest that during meiosis the *MAT* locus may be exchanged onto different genetic backgrounds and therefore have broad evolutionary implications with respect to mating-type switching in both model and and pathogenic yeasts.

77. **Survey of mating-type *(MAT)* gene distribution in the Penicillia.** Carly Eagle[1], Rob A. Samson[2], Paul S. Dyer[1] [1]School of Biology, University Park, University of Nottingham, Nottingham, NG7 2RD [2]Fungal Biodiversity Centre, CBS, P.O. Box 85167, 3508 Utrecht, The Netherlands sbxcee@nottingham.ac.uk

The genus *Penicillium* contains various species that are important in medicine, industry and food spoilage. This genus is polyphyletic, containing four subgenera; *Biverticillium, Furcatum, Aspergilloides* and *Penicillium*. Studies are underway to investigate the genetic basis of reproductive mode in the Penicillia, initially involving a screen of mating-type *(MAT)* genes in asexual and sexual taxa. *Penicillium marneffei* has previously been shown to contain either a *MAT1-1* or *MAT1-2* locus. This species is classified within the subgenus *Biverticillium* which is closely related to the sexual genus *Talaromyces*. The focus of current work is the subgenus *Penicillium* which contains the asexual species *Penicillium chrysogenum* (produces penicillin) and *P. griseofulvum* (produces griseofulvin), and is closely related to the sexual genus *Eupencillium*. Using a degenerate PCR approach, *MAT1-1* and *MAT1-2* loci have been amplified and sequenced from a number of species belonging to this subgenera.

78. **Reproductive strategy and genetic polymorphism in *Pleurotus pulmonarius* populations in Russia.** Alla Shnyreva. Department of Mycology and Algology, Moscow State University, Moscow, Russia, e-mail: shnyreva@herba.msu.ru

The genetic structure of populations of *P. pulmonarius* was inferred from variation among 157 individuals at 14 polymorphic allozyme loci and RAPD-profiles to determine the extent of geographic differentiation in this widespread mushroom species in Central Russia. Population genetic analysis was carried out by employing homokaryotic and dikaryotic strains (parents – offspring). To define interbreeding fungal populations, crosses between homokaryons were performed. Genetic exchange was shown to be not only limited to the sexual cycle and to occur in heterokaryon- homokaryon mating (he-ho-mating). The majority of the genetic variation was contained within populations; however, considerable genetic differentiation was observed among populations from three distant regions (F_{ST} = 0.750). The low level of inbreeding (F_{IS} = 0.018) suggested that the populations were panmictic. The most common type of mating events was confirmed to be a spore-mediated outcrossing reflected in spatial distribution of mating type factors. However, gene flow between geographic localities was restricted. Clustering analysis demonstrated that genetic distance was correlated with geographic distance and that a large component of the genetic variation was due to allele frequency differences among populations. Population structure of *P.pulmonarius* will be discussed with a special reference to sexual reproduction, clonality, and outcrossing events. The research was supported by RFBR grant 06- 04- 08161.

79. **Watching crystal balls: Asexual life-cycles and morphology of cultured mesomycetozoans.** Wyth Marshall[1], Gail Celio[2], Mahajabeen Padamsee[2], David McLaughlin[2], Mary Berbee[1]. [1]Department of Botany, University of British Columbia, Vancouver, B.C. [2]Department of Plant Science, University of Minnesota, St. Paul, MN. WythMarshall@Yahoo.ca

Several clades of unicellular organisms diverged near the animal/fungal divide. Of these, the Mesomycetozoans or Ichthyosporids are perhaps the most diverse and are comprised of approximately 70 spp. of animal commensuals and parasites. Mesomycetozoans have been isolated from animals ranging from arthropods and molluscs to vertebrates but very few have been propagated in culture. We have successfully cultured five new species isolated from the gut contents of various marine invertebrates. We characterized the species using ribosomal DNA sequences and have studied their life- cycles and ultrastructure using microscopy. We documented and photographed four mechanisms of dispersal and the asexual life-cycles of three species. One species, which we plan to name '*Creolimax fragrantissima*,' disperses via numerous slug-shaped amoebae and produces multicellular clusters. Further details of amoeboid behaviour, phylogenetic placement and ultrastructure of this new species will be presented. Understanding of the biology of these closely related fungus-like protists is integral to our understanding of how fungus-like forms evolved and why the true fungi became such a successful multicellular kingdom.

80. **Evolutionary Aspects of the Alkaloid Biosynthesis in *Claviceps* sp.** Nicole Lorenz[1], Caroline Machado[2], Ella Konnova[2], Chris Schardl[2], Paul Tudzynski[1]. [1]Institute of Botany, WWU Muenster, Germany. [2]Department of Plant Pathology, University of Kentucky, USA

The genus *Claviceps* consists of many phytopathogenic ascomycets producing ergot alkaloids during a specific stage of their lifecycle. Ergot alkaloids show high homology to some neurotransmitters and bind to the same receptors in the CNS leading to an application in a variety of clinical conditions. The genes encoding for the enzymes needed for alkaloid production are organized in a cluster. To study the evolution of the alkaloid biosynthesis the work focused on a comparison of the cluster sequences of three *Claviceps* species producing different kinds of alkaloids as endproducts of their pathways. While *C. purpurea* is able to synthesize ergopeptides the other species fulfill only a truncated pathway leading to an accumulation of species-specific intermediates. *C. fusiformis* shows a corresponding cluster including the genes for the early steps within the pathway. Two additional genes for further synthesis are present but complementation of the corresponding knock-out mutants of *C. purpurea* failed (the genes seem to be not functional due to a rearrangement event) whereas the implementation of a functional gene of *C. purpurea* results in a modified alkaloid spectrum of *C. fusiformis*. *C. hirtella* synthesizes ergometrine, a simple ergopeptide and should only lack the large NRPS genes. First sequence analysis confirmed these assumptions.

81. **Rapid evolution by genetic decay of mating type (*mat*) genes in homothallic *Neurospora***. Lotta Wik, Magnus Karlsson, Hanna Johannesson Dept of Evolutionary Biology, Uppsala University Hanna.Johannesson@ebc.uu.se

In filamentous ascomycetes, three basic sexual reproductive modes are present: heterothallism, homothallism and pseudohomothallism. The *mat* locus is the sole determinant of the mating type in *Neurospora*, and is the master-regulator of sexual reproduction in this genus. We sequenced the four *mat*-genes from *Neurospora* taxa with different reproductive systems, and used likelihood-based methods to reveal that two *mat*-genes (*mat a-1* and *mat A-2*) evolve under positive selection in heterothallic taxa, while all four *mat*-genes in homothallic taxa are subjected to an evolutionary decay. In all four genes a lower ratio of non-synonymous to synonymous substitutions (dN/dS) was found in heterothallic than in homothallic taxa. This is due to a higher proportion of conserved sites in the heterothallic dataset and a higher proportion of neutrally evolving sites among the homothallic species. While the heterothallic taxa show a codon or domain specific selective pressure, the codons with elevated dN/dS were more evenly distributed along the genes in the homothallic dataset. This indication of *mat*-gene degeneration is further supported by the observations of a disrupted ORF of *mat a-1*, *mat A-2* and *mat A-3* in homothallic taxa, and the finding of *mat A-2* being silenced in *N. africana* and *N. galapagosensis*, thus becoming a pseudogene in these two taxa. Our result indicate that *mat*-genes evolve in an adaptive manner in heterothallic taxa, while in homothallic taxa they are not needed to be functionally conserved.

82. **Many Globally Isolated AD Hybrid Strains of *Cryptococcus neoformans* Originated in Africa.** Anastasia P. Litvintseva, Irka Templeton, Joseph Heitman, and Thomas G. Mitchell. Duke University Medical Center, Durham, USA

AD strains of *C. neoformans* are hybrids between *C. neoformans var. grubii* (serotype A) and *C. neoformans var. neoformans* (serotype D). While most isolates of serotype A and D are haploid, AD strains are diploid or aneuploid, contain two sets of chromosomes and two mating type alleles, MATa and MATalpha, one from each of the serotypes. The global population of serotype A is dominated by isolates with the MATalpha mating type; however, about half of the globally analyzed AD strains possess the extremely rare serotype A MATa allele. Previously we described an unusual population of serotype A in Botswana, in which 25% of the strains contain the rare MATa allele. Here we utilized two methods, phylogenetic analysis of three genes and genotyping by AFLP, and discovered that AD hybrid strains possessing the rare serotype A MATa allele are clonally related and cluster with isolates of serotype A from Botswana, whereas AD hybrids that possess the MATalpha serotype A allele cluster with cosmopolitan isolates of serotype A. We also determined that AD hybrids are more resistant to UV radiation than haploid serotype A strains from Botswana. These findings support two hypotheses: (i) AaDalpha strains originated in Botswana from a cross between strains of serotype A and D; (ii) This fusion produced strains with increased fitness, enabling the Botswanan serotype A MATa genome, which is otherwise geographically restricted, to propagate globally.

83. **Horizontal gene transfer as the source of extra genes in *Aspergillus oryzae*. Nora Khaldi[*], Kenneth H. Wolfe.** khaldin@tcd.ie *Smurfit Institute of Genetics. University of Dublin Trinity College Dublin 2. Ireland.*

Although horizontal gene transfer (HGT) is a major aspect of prokaryotic evolution and is becoming increasingly documented between prokaryotes and eukaryotes, very few cases of HGT of nuclear genes from eukaryote to eukaryote have been documented so far. Several obstacles impede the unequivocal demonstration of HGT. The detection of foreign genes is facilitated if the recipient and donor genomes are from two different kingdoms, but if they are relatively closely related then observations suggestive of HGT may be equally consistent with alternative scenarios of gene duplication and gene loss. *Aspergillus oryzae* is a filamentous fungus that has a large gene complement compared to its annotated relatives. Here we test three alternative explanations for the existence of these extra genes in *Aspergillus oryzae*: recent gene duplication, whole genome duplication, and HGT. We show using gene location, evolutionary rate, and phylogeny based methods that HGT took place from the close sister subphylum *Sordariomycetes* into an ancestor of *Aspergillus oryzae*. Finally, we investigate the role of the transferred genes and show that transfer played a role in enriching Aspergillus oryzae's gene repertoire, specifically by expanding its hydrolytic enzyme gene complement.

84. **The influence of CAT fusion on colony establishment in *Neurospora crassa***. M. Gabriela Roca [1*], Natalia Angarita-Jaimes[2], Catherine Towers[2], David Towers[2] and Nick Read[1] 1 Institute of Cell Biology, University of Edinburgh, UK 2 School of Mechanical Engineering, University of Leeds, UK * Gabriela.Roca@ed.ac.uk

Hyphal network formation is a key feature of filamentous fungi. We are using *Neurospora crassa* as a model to study hyphal networks which are formed during colony establishment by means of conidial anastomosis tube (CAT) fusion. The role of CAT fusion in colony establishment is presently unclear. We have addressed this issue by recording germling growth and nuclear dynamics during the first 10 h following conidial germination. Nuclei were labeled with H1::GFP and imaged by confocal microscopy. Advanced image processing techniques were used to track nuclear motion and determine germ tube growth rates. Nuclei were identified by a particle mask correlation method and then tracked with a particle tracking velocimetry (PTV) algorithm. Germling growth was monitored by means of a level set algorithm for centerline extraction. Germ tubes within microcolonies derived from single conidia grew significantly more slowly (< 0.03 ìm/sec) than colonies formed from conidia

that had fused via CATs (< 0.085 ìm/sec). Nuclei in microcolonies derived from fused conidia achieved higher velocities (< 1.2 ìm/sec) than those formed from single unfused conidia (< 0.8 ìm/sec). Our initial results provide evidence for cooperative behavior between fused conidial germlings; CAT fusion seems to stimulate germ tube growth and actively promote nuclear mixing.

85. **Molecular characterization of the *Fusarium graminearum* species complex in Japan**. H. Suga[1], G.W. Karugia[1], T. Ward[2], L.R. Gale[3], K. Tomimura[4], T. Nakajima[4], A. Miyasaka[4], S. Koizumi[4], K. Kageyama[1], and M. Hyakumachi[1]. [1]Gifu University, Gifu, Japan. [2]Agricultural Research Service, USDA, Peoria, USA. [3]University of Minnesota, St. Paul, USA. [4]National Agricultural Research Center, Tsukuba Japan.

We collected 298 strains of the the *Fusarium graminearum* species complex from wheat or barley from 2001 to 2004 in Japan and investigated species and trichothecene chemotype compositions. Species-diagnostic PCR-RFLP revealed the presence and differential distribution of *Fusarium graminearum* s. str. and *Fusarium asiaticum* in Japan. *F. graminearum* s. str. is predominant in the north, while *F. asiaticum* is predominant in southern regions. In the Tohoku area, distinct co-occurrence of these species was observed. Chemotyping by multiplex PCR revealed significantly different chemotype compositions of these species. All 50 strains of *F. graminearum* s. str. were of a 15- or 3-acetyl deoxynivalenol type, while 173 out of 246 strains of *F. asiaticum* were of a nivalenol type. The possibility of gene flow between the two species was investigated by PCR-RFLP markers, as interspecies hybridization of *F. graminearum* s. str. and *F. asiaticum* was previously achieved *in vitro*. However, no obvious hybrids were detected from 98 strains examined, including strains collected from regions where both species co-occur.

86. Withdrawn

87. ***Phytophthora* database: An integrated resource for detecting, monitoring, and managing *Phytophthora* diseases.** Seogchan Kang[1], Jaime Blair[1], Dave Geiser[1], Izabela Makalowska[1], Sook-Young Park[1], Bongsoo Park[1], Mike Coffey[2], Kelly Ivors[3], Yong-Hwan Lee[4], Jong-Seon Park[4], and Frank Martin[5]. [1]Penn State, University Park, PA. [2]UC, Riverside, CA. [3]NCSU, Raleigh, NC. [4]Seoul National Univ., Seoul, Korea. [5]USDA-ARS, Salinas, CA.

High virulence of *Phytophthora* species and their ability to spread rapidly establishes *Phytophthora* as one of the most important groups of plant pathogens. The ability to accurately and rapidly identify the causal agent of a disease is crucial for developing effective regulatory and management strategies against *Phytophthora*. The *Phytophthora* database project (Link here) was initiated to enhance our capability of rapid detection and diagnosis of *Phytophthora* spp. by archiving known genotypic and phenotypic diversity in a highly integrative database. To compliment the species morphological descriptions and serve as a molecular reference for isolate identification, ~2000 accessions representing 71 morphological species maintained in the WPC Genetic Resource Collection have been genetically characterized. A seven-locus phylogeny of the whole genus supports the division of *Phytophthora* into approximately eight major groups. In addition to these nuclear genes, four mitochondrially encoded genes are being sequenced from the same isolates to construct a mitochondrially based phylogenetic framework. This project is on-going; sequence and species data are continually being deposited, and new data analysis and visualization tools are being developed to increase utility and breadth of this database. An overview of database functions will be presented.

88. **A novel kind of cooperation facilitates conidial germination in *Neurospora crassa***. Franck Richard [1], N. Louise Glass [2], and Anne Pringle [1]. [1] Harvard University, Organismic and Evolutionary Biology, 16 Divinity Avenue, Cambridge MA 02138. [2] University of California Berkeley, Plant and Microbial Biology, 111 Koshland Hall, Berkeley CA 94720 pringle@oeb.harvard.edu

The cooperative behaviors of filamentous fungi have long fascinated mycologists. In these experiments we use micromanipulations of individual conidia to demonstrate that time to germination is significantly reduced when two or more conidia are grouped. Single conidia take longer to germinate and both the length of the germ tube, as well as the perimeter of a colony, are significantly smaller as compared to germ tubes and colony perimeters of two or more conidia. A similar result is found with two wildtype strains as well as the *soft* mutant (which lacks the ability to fuse), proving that the pattern is a general one and is likely to be controlled by early signals working independently of the fusion process. Experiments with isolates from geographically distant locations suggest that this kind of cooperation functions even when isolates are genetically distinct, but whether one isolate is co-opting the mechanism for its own benefit remains to be tested.

89. **Interspecific transfer of host-specific toxin genes in *Stagonospora nodorum.*** Richard P Oliver[1], Peter S. Solomon[1], James Hane[1], Eva H. Stukenbrock[2], Bruce A McDonald[2], Zhaohiu Liu[3], Justin D Faris[3], Timothy L Friesen[3]. [1]ACNFP, Murdoch University, Australia [2]ETH, Zurich, Switzerland [3]USDA and NDSU, ND

The host-specific toxin ToxA, produced by *P. tritici-repentis*, confers virulence on wheat genotypes carrying Tsn1. The *Stagonospora nodorum* gene, SnToxA was identified from a reannotation of the genome sequence and is nearly identical to

PtrToxA. Disruption of SnToxA reduced disease on wheat lines carrying Tsn1. ToxA genes from a large isolate collection from *P. tritici-repentis* were identical but those from *S. nodorum* were highly variable. *P. tritici-repentis* was first identified in 1902 but it was not until 1941 that typical tan spot symptoms were first described. Nowadays, *P. tritici- repentis* is a regular and abundant pathogen. Our evidence strongly suggests that ToxA was horizontally transferred to *P. tritici-repentis* some time prior to 1941. These new forms appear to have rapidly spread around the world, probably in grain shipments (Nature Genetics 38 953-956). Our work indicates that *S. nodorum* produces several other toxins. The presence of multiple host-specific toxins and their transfer between species raises many interesting evolutionary questions; 1. How does toxin possession and expression affect the fecundity of fungal strains carrying the toxins? 2. How can we explain the expression of toxin receptor genes? 3.What are the mechanisms of sequence diversification in toxin genes?

90. **Explosively launched ascospores are shaped to minimize drag.** Marcus Roper, Michael P. Brenner and Anne Pringle, Harvard University Cambridge MA 02138, pringle@oeb.harvard.edu

Many species of ascomycete fungi have a sexual phase in which ascospores are forcibly ejected clear of the fruiting body. Reproductive success is dependent upon the spores germinating away from the parent fungus, and thus the spores must be ejected at very large launch speeds in order to pass through the layer of still air surrounding the fruiting body, into the vigorous air currents beyond. To do this these fungi have evolved mechanisms for applying initial accelerations that are unmatched in the plant or animal kingdoms. Spores are tiny, fast moving objects, so the distance that they can travel in still air is severely limited by fluid drag. We present evidence that drag minimisation is a primary determinant of spore shape. This results leads to several predictions, including upper and lower bounds upon spore size (spores are predicted to be no smaller than 1.8 micrometers and no larger than 30 micrometers) and a constant launch speed of 2 m/s across the phylum.

91. **Sexual mating of *B. cinerea (fuckeliana)* illustrates PRP8 intein HEG activity.** Annika A.M. Bokor and Russell T.M. Poulter, University of Otago, Dunedin, New Zealand

Inteins are proteins that are removed post-translationally from a host protein. This is an autocatalytic splicing process necessary for the maturation of the host protein. Inteins typically contain not only the necessary splicing domains but also a homing endonuclease (HEG). The HEG enables the intein encoding sequence to spread through the gene pool of a species and potentially to also undergo horizontal transmission to other species. In a diploid, heterozygous for the presence/absence of the intein gene, the HEG recognises a 30-50bp target sequence at the 'empty' allelic site. It introduces a double-stranded DNA break at this target. This double-strand break triggers the host's recombinational repair system, which in the repair process copies the full-length intein into the target site. Our group has discovered several nuclear full-length inteins (located in the critical PRP8 gene) in species including the human fungal pathogens *Aspergillus fumigatus, Histoplasma capsulatum* and the plant pathogen *Botrytis cinerea*. To demonstrate intein replication a species must have a sexual cycle and there must be polymorphism for presence/absence of the intein in the gene pool of the species. We have demonstrated that *B. cinerea* is polymorphic for presence/absence of the PRP8 intein. Characterisation of HEG activity in *B. cinerea* sexual cycle will be presented. This is the first mycelial system in which the intein replication cycle has been demonstrated.

92. **Global molecular epidemiology of *Cryptococcus gattii* VGII isolates traces the origine of the Vancouver Island outbreak to Latin American.** Wieland Meyer [1], Sirada Kaocharoen [1], Luciana Trills [2], Alexandro Jover-Botella [1], Patricia Escandón [3], Elizabeth Castañeda [3], Clement Tsui [1], Ferry Hagen [4], and Teun Boekhout [4]. [1] Molecular Mycology Research Laboratory, CIDM at Westmead Hospital, Westmead Millennium Institute, The University of Sydney Western Clinical School, Westmead, NSW, Australia. [2] Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil. [3] Grupo de Microbiología, Instituto Nacional de Salud, Bogotá, Colombia. [4] Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

The basidiomycetous yeast *Cryptococcus gattii* causes life-threatening disease mainly in immunocompetent patients. Four molecular types have been identified: VGI/AFLP4-VGIV/AFL7. Two subtypes of molecular type VGII/AFLP6 have emerged in 2000 as a primary pathogen on Vancouver Island, Canada, causing a large scale cryptococcosis outbreak in humans and animals: VGIIa/AFLP6A, the major and more virulent and VGIIb/AFLP6B, the minor and less virulent genotype. A global molecular epidemiological study of more than 160 VGII strains recovered since 1986 was conducted using PCR fingerprinting, AFLP and MLST analysis (8 polymorphic loci: ACT1, CAP59, IDE, IGS, LAC1, PLB1, SXI1alpha, URA5). VGIIa is also present in Colombia, Brazil, Venezuela, Argentina, USA, Thailand and Greece. Australian VGII isolates clustered with VGIIb. Brazilian VGIIa isolates are identical with the Vancouver Island outbreak strains. Colombian VGIIa isolates are closely related but not identical to the Vancouver Island outbreak strains and are avirulent. The vast majority of Colombian isolates are MATa compared with the Vancouver Island outbreak isolates (all MATalpha). In vitro mating experiments showed that the avirulent Colombian MATa strains mate with the virulent VGII MATalpha strains from Brazil and Vancouver. The fact that there are VGIIa and VGIIb isolates recovered as early as in 1986 in South America indicates that these genotypes may have been present for a long time in the Americas rather than being a result of a recent recombination event between a less virulent genotype introduced to North America from Australia and an unknown mating partner as suggested by Farser et al. (Nature 437,2005).

93. **Evolution and phylogeny of *Candida* species inferred from multigene analyses**. Clement Tsui [1], Heide-Marie Daniel [3], Wieland Meyer[1,2]. [1]Molecular Mycology Research Laboratory, Center for Infectious Diseases and Microbiology, Westmead Hospital, Westmead, Australia; email: clementsui@gmail.com [2]Western Clinical School, Department of Medicine, The University of Sydney, Australia; [3]BCCM/MUCL culture collection, Université catholique de Louvain, Unite de Microbiologié, Louvain-la- Neuve, Belgium.

Candida species are ubiquitous human pathogens, plant endophytes and insect symbionts. Phylogenies based on rDNA and actin sequences suggested that the genus is not monophyletic, and did not clearly resolved the relationships among related genera and species. Protein coding genes are useful to resolve taxonomic positions among a broad range of fungi. We selected over 70 taxa from *Candida* and allied genera, and investigated their phylogenetic relationships using nuclear sequences of the largest subunit (RPB1) and second largest subunit (RPB2) of RNA polymerase II gene, actin, mitochondrial cytochrome oxidase subunit 2 (COX2) gene, and LSU rDNA. The DNA sequences were subjected to maximum parsimony and Bayesian analyses. Four major phylogenetic groups were recognized according to Bayesian probabilities. Group A contained six pathogenic species of *Candida*, seemed to derive from non-pathogenic species, while Group B contained species of *Clavispora*, *Metschinikowia*, and *Pichia guilliermondii*. Species of *Debaryomyces* were related to groups A and B but formed an independent clade. Group C consisted species in the Saccharomycetaceae, in sibling relationship with a clade containing *Pichia jadinii*. *Pichia fermentans* and other environmental species concentrated in Group D. The phylogenetic relationships of species and the evolution of codon usage would be discussed.

94. **Phylogeny of clusters of genes with predicted roles in biosynthesis of epipolythiodioxopiperazine toxins in ascomycetes**. Nicola Patron[1], Anton Cozijnsen[1], Bill Nierman[2], Ross Waller[1] and Barbara Howlett[1]. [1]School of Botany, the University of Melbourne, Australia; [2] The Institute for Genomic Research, USA

Epipolythiodioxopiperazines (ETPs) are toxins made by phylogenetically diverse filamentous fungi. Ten clustered genes are responsible for biosynthesis of the core ETP moiety. Clusters with most or all the core genes are present in *Magnaporthe grisea, Chaetomium globosum, Fusarium graminaerum, Trichoderma virens, T.reesei, Leptosphaeria maculans, Penicillium lilacinoechinulatum, Aspergillus terreus, A.clavatus, A.flavus, A.oryzae, A.fumigatus, Neosartorya fischeri*. Each of six cluster genes examined is closely related phylogenetically to non-cluster paralogues within filamentous fungi. Similar relationships were inferred for all six genes, each one sharing a common ancestry to the exclusion of non-cluster paralogues, excepting cluster genes from *C.globosum* and *F.graminarium*. This suggests that most ETP gene clusters have a common origin and that the cluster was inherited relatively intact, rather than assembling independently in the different ascomycete lineages. Internal topologies of clusters do not reflect phylogeny of ascomycete lineages. Gene clusters fall into three phylogenetic classes, which do not necessarily reflect the structures of toxins produced. The disjunct heredity of these clusters may arise from multiple independent losses of cluster class or lateral gene transfer of clusters between lineages.

95. **Horizontal Gene Transfer and Recombination in Histone 3 Gene Evolution of Alternaria.** Soon Gyu Hong, and Barry M. Pryor. Korea Polar Research Institute, Incheon, Korea.

Evolution of H3 gene was analyzed by comparing H3 gene phylogeny with reference phylogeny constructed by alt a 1, gpd, and ITS sequences in *Alternaria* and related species. Several H3 gene types defined by intron presence and absence were recovered and distribution of each gene type on the phylogeny was very complex. We postulated four alternative hypotheses to explain the findings: 1) maintenance of different types of H3 gene in a strain with one of them as a major copy and the others as minor copies; 2) repeated insertion and deletion of introns during evolution of H3 genes; 3) maintenance of multiple copy of the gene in the ancestral species and independent loss of different type of genes in each descendent; 4) exchange of genetic materials among strains leading to horizontal transfer of the gene and recombination between different copies of the gene to achieve concerted evolution. Among them, the fourth hypothesis of horizontal gene transfer and recombination was chosen as the most plausible explanation for the evolution of H3 gene in *Alternaria* and related species. The first hypothesis of maintaining multiple types of H3 gene could explain the multiple PCR bands of radicina species-group and *Stemphylium* group.

96. **Extent of Genetic Diversity in a Single Fruiting Body of *Parmelia laevior.*** Jin Sung Lee and Soon Gyu Hong. Korea Polar Research Institute, Incheon Korea.

There have been debates in defining species boundary in fungi with emphasis on phenotypic or genotypic characteristics. To test the extent of genetic diversity in lichen-forming fungus, *Parmelia laevior*, sequence variations of three genes (ITS, RPBII and mtSSU rDNA) were analyzed from 20 spore-germinated cultures from an identical fruiting body. All of the isolates contained identical mtSSU rDNA sequences, whereas two types of ITS and RPBII sequences were observed. Two types of ITS sequences showed 7 nucleotide difference among 639 bases (98.9% similarity). Two types of RPBII sequences showed 16 nucleotide difference among 1192 bases (98.83% similarity). To discriminate mixed cultures from homogeneous single spore cultures, two types of single-copy RPBII gene was specifically detected by oligonucleotide ligation assay (OLA) based upon the single nucleotide polymorphism. As a result, 13 isolates turned out as mixed cultures by showing both types of RPBII sequences. To

detect incomplete concerted evolution of ITS sequences, OLA was applied for ITS sequences and it was found that all of the single-spore isolates had only one type of ITS sequences. RPBII and ITS alleles were segregated together in all of the single-spore isolates, implying that they are closely linked on the chromosome. From the identical sequences of mtSSU rDNA and high variation of ITS and RPBII sequences, it is concluded that boundary of species in *Parmalia laevior* is broader than have been expected from previous researches.

97. **The evolution of mating type idiomorphs in the genus *Mycosphaerella***. Mahdi Arzanlou[1,2], Lute-Harm Zwiers[1], and Pedro W. Crous[1,2]. [1]Evolutionary Phytopathology Group, CBS Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. [2]Laboratory of Phytopathology, Wageningen University, Binnenhaven 5, 6709 PD Wageningen, The Netherlands. arzanlou@cbs.knaw.nl

The genus *Mycosphaerella* is one of the largest genera of ascomycetes, comprising several thousand species. Despite the diverse ecological habitats inhabited by species of *Mycosphaerella*, many species are specialized plant pathogens, occurring on a wide range of hosts ranging from monocots, dicots to even gymnosperms. The fact that different *Mycosphaerella* species can co-occur on a single host and even within a single lesion, as well as their ability to jump between hosts, makes *Mycosphaerella* a good model to study the (co) evolution of closely related species. The mating strategy for the majority of *Mycosphaerella* species is yet unknown; for a few species a heterothallic or homothallic mating system has been observed. However, for the majority of the *Mycosphaerella* species the understanding of sex remains a puzzle. A large number of species in this genus are known only from their teleomorphs, while for many other species no *Mycosphaerella* state has been discovered, and only the anamorphic stage is known. In order to examine the importance of sexual reproduction in evolution and speciation of *Mycosphaerella* species occurring on banana, we analyzed the structure of the mating type idiomorphs of several additional *Mycosphaerella* species. Analysis of the structure of the mating type genes from homothallic and heterothallic members of this genus showed unique features in the mating type loci. In all mating type idiomorphs additional open reading frames ("ORFs") can be distinguished which are seemingly unique to the *Mycosphaerellaceae*. Furthermore, the obtained data suggest that heterothallism is in most cases the ancestral state, and evidence exists that that some heterothallic species have evolved from a homothallic ancestor.

98. **Molecular Analysis and Evolution of Gibberellin Biosynthetic Gene Clusters in Fungi**. Christiane Bömke and Bettina Tudzynski. WWU Münster, Institut für Botanik, Schlossgarten 3, Germany

Although gibberellins (GAs) are ubiquitous diterpenoid plant hormones, they were first identified as secondary metabolites of *Fusarium fujikuroi*. Today it is known that they are also produced by several bacteria and some other fungi. Since a horizontal gene transfer of the GA genes from plants to fungi can be excluded due to fundamental differences at the chemical (pathway), biochemical (enzymes) and genetic level, we are interested in the origin of the fungal GA cluster genes. To elucidate this question, we focus on the distantly related GA producing fungus *Sphaceloma manihoticola*. The spectrum of the GAs present suggests that the GA biosynthesis in *Sphaceloma* follows the pathway known for *Fusarium*. However, in contrast to *F. fujikuroi*, the biosynthesis does not go to the end product GA_3 but stops two steps earlier with GA_4. The assumption that the responsible genes (*P450-3* and *des*) are missing was confirmed after cloning of the *S. manihoticola* GA gene cluster: homologues of *P450-3* and *des* which flank the gene cluster in *Fusarium* are missing in this cluster. The order of the other five genes is similar but not identical to *F. fujikuroi* due to gene inversions. The functional analysis of the genes was done by a combination of knock-outs and complementation of the corresponding *F. fujikuroi*-mutants. A heterologous gene expression of *Ff-P450-3* and *Ff-des* will show if the complete pathway can be restored.

99. **An Unprecedented Shift in the Composition of Fusarium Head Blight Pathogens in North America due to the Rapid Spread of a Highly Toxigenic Population of *Fusarium graminearum*.** Todd J. Ward[1], Randall M. Clear[2], Alejandro P. Rooney[1], Kerry O'Donnell[1], Don Gaba[2], Jeannie Gilbert[3], Susan Patrick[2], and David E. Starkey[4]. [1]Microbial Genomics and Bioprocessing Research Unit, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois, USA. [2]Grain Research Laboratory, Canadian Grain Commission, Winnipeg, Manitoba, Canada. [3]Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada. [4]University of Central Arkansas, Conway, Arkansas, USA.

To date, a fraction of the combined species and chemotype diversity of Fusarium head blight (FHB) pathogens is present in North America. However, our active surveillance with a unique multilocus genotyping assay revealed for the first time that 3ADON-producing isolates of *F. graminearum* are prevalent in North America. In addition, we identified a dramatic East-West (3ADON-15ADON) chemotype cline in Canada, and documented a recent and unprecedented shift in FHB pathogen composition in North America by demonstrating that the 3ADON chemotype frequency in Canadian prairie provinces increased 14-fold between 1998 and 2004. Analyses of population structure based on nine VNTR markers demonstrated that 3ADON and 15ADON isolates from western Canada represent distinct populations ($F_{ST} = 0.4$, $P < 0.001$) with low levels of gene flow evidenced by the identification of a small number of admixed isolates. On average, isolates from the 3ADON population produce significantly ($P < 0.001$) higher levels of trichothecene toxins, grow faster, and produce more spores *in vitro* than isolates from the 15ADON population. Combined with the rapid and significant shift in chemotype frequency that we have documented, these data suggest that a recently introduced or emergent population of *F. graminearum* with a 3ADON chemotype has a selective advantage and is

rapidly changing the FHB landscape in North America. In addition, the demonstration that highly toxigenic FHB pathogens with a novel trichothecene profile have rapidly displaced less toxigenic isolates has significant implications for food safety and disease control efforts.

100. **Profiling Fungal Diversity in Green Lake.** Heather Michael, Michael L. McCormick, Jinnie M. Garrett. Department of Biology, Hamilton College, Clinton, NY, USA. jgarrett@hamilton.edu

Exhibiting permanent geochemical stratification, Green Lake near Fayetteville New York, is an example of a meromictic lake. The lake's physical environment is structured like a layer-cake: two chemically distinct ecozones— an upper oxic mixolimnion and lower anoxic monimolimnion— remain separated by a stable redox transition zone, or "chemocline". Previous diversity studies have been conducted at other meromictic lakes. While related research has focused on bacterial and archaeal assemblages; few, if any, attempts have been made to characterize fungal diversity in meromictic environments. The goal of the present study is to determine how fungal diversity in Green Lake varies with depth and the associated changes in water chemistry. Our geochemical analysis revealed distinct stratification, with dramatic shifts in pH, temperature, oxidation-reduction potential, and specific conductance occurring within the chemocline. We expect that niche differentiation and community structure will exhibit vertical stratification in association these changes in water chemistry. In June of 2005, Microbes were collected from Green Lake via sterile syringes deployed at 23 depths spanning the chemocline. Sampling intervals varied from 5 m in chemically uniform regions of the mixolimnion and monimolimnion to 25 cm across the chemocline. A 500bp region of fungal 18s rDNA was selectively amplified using taxon-specific primers. We are currently in the process of using Denaturing Gradient Gel Electrophroesis (DGGE) to profile fungal diversity from water samples collected at various depths. Following DGGE, unique 18s rDNA amplicons will be sequenced to determine community composition. Preliminary DGGE of a chemocline community indicates a richness of 8 to 12 fungal species. Depth-related trends in fungal diversity will be presented.

101. **Structural and Functional Evolution of Non-Ribosomal Peptide Synthetases.** Kathryn E. Bushley[1] and B. Gillian Turgeon[1]. [1]Department of Plant Pathology, Cornell University.

Non-ribosomal peptide synthetases (NRPSs) are multimodular enzymes that make non-ribosomal peptides (NRPs) through a thiotemplate mechanism independent of ribosomes. These structurally diverse molecules have important roles in basal and niche-specific developmental success of filamentous fungi. In addition to roles they play in their producers' lives, NRPs have diverse biological effects on other organisms including antibiotic, immunosuppressant, antitumor and virulence-promoting activities. Previous studies suggested that NPS genes have a highly discontinuous distribution in filamentous ascomycetes (Lee et al., Eukaryotic Cell, 2005). Comparative analyses of a larger dataset suggest that NPSs show varying levels of functional and structural conservation. Genes with functions critical to the fungal cell (stress resistance, involvement in sexual and asexual reproduction, virulence, morphology, etc) are highly conserved. For example, *Cochliobolus heterostrophus* NPS6, responsible for extracellular siderophore biosynthesis (Oide at al., Plant Cell, 2006) is important for virulence and resistance to oxidative stress and is conserved both structurally and functionally across all fungi examined to date. Homologs of ChNPS2, responsible for biosynthesis of an intracellular siderophore, are also found in most fungi but show a history of duplication into two clades, NPS2 and NPS1/SidC/Sid2 (represented by *Fusarium graminearum* NPS1/*Aspergillus nidulans* SidC/*Ustilago maydis* Sid2), with corresponding differences in function. At the other end of the spectrum are taxon-specific genes such as ChNPS1 and ChNPS3 which are intact in only a few closely related *Cochliobolus* species. We are using a variety of phylogenetic tools to address the roles of recombination, natural selection, and duplication/loss, in generating diversity of NPSs and their corresponding peptide products.

102. **Evolution of the *Cryptococcus* Mating-type Locus: a Comparative Genomics Approach**. Keisha Findley[1], James Fraser[2], and Joseph Heitman[1].Department of Molecular Genetics and Microbiology[1], Duke University, Durham, North Carolina, School of Molecular and Microbial Sciences[2], University of Queensland, Brisbane, Australia. kmf13@duke.edu

Many basidiomycetous fungi have tetrapolar mating-type systems in which mating is regulated via two unlinked loci that control and establish cell identity and both must differ for sexual reproduction. One *MAT* locus encodes pheromones and pheromone receptors, while the other *MAT* locus encodes homeodomain transcription factors. In contrast, the fungus *Cryptococcus* exhibits a bipolar mating system with a single locus that encodes the pheromones, receptors, and transcription factors that establish mating-type. The ability of this fungus to reproduce sexually has been studied extensively and underlies development and is linked to pathogenicity. To characterize the evolution of the mating-type locus of this pathogenic fungal species complex, a comparative genomics approach is being applied with distantly and closely related species. This analysis involves cloning and sequencing the *MAT* locus from the closely related species *Tsuchiyaea wingfieldii*, *Filobasidiella depauperata*, *Bullera dendrophila*, *Cryptococcus amylolentus*, *Cryptococccus heveanensis* and the more distantly related species *Cryptococcus laurentii* and *Cryptococcus albidus*. To identify genes associated with *MAT* in these species, degenerate primers are applied for two genes flanking *MAT* (*FAO1* and *NOG2*) and two highly conserved genes within *MAT* (*RPO41* and *LPD1*). High-density filters of genomic fosmid libraries are produced and probed with these genes to identify *MAT*-specific fosmids. Positive clones are confirmed by Southern analysis and pooled to generate sequencing libraries. Thus far, preliminary assemblies have been

generated for *T. wingfieldii* and *C. heveanensis*. Our results reveal that homologs of the homeodomain proteins Sxi1α and Sxi1a are both present and divergently transcribed similar, to bE and bW in *Ustilago maydis*. A second unlinked gene cluster encodes pheromone and pheromone receptor homologs and many other *MAT* associated genes. This analysis reveals that the translocation event that collapsed a tetrapolar to a bipolar system, and loss of one or the other homeodomain genes, occurred recently, perhaps concomitant with the emergence of this pathogenic species complex. Further *MAT* sequencing and studies on sexual reproduction potential are in progress.

103. **Cryptic Sexuality in *Aspergillus parasiticus* and *A. flavus***. Jorge H. Ramirez-Prado[1], Geromy G. Moore[1], Bruce W. Horn[2], Ignazio Carbone[1]. [1]Center for Integrated Fungal Research, Department of Plant Pathology, North Carolina State University, Raleigh, NC. [2]National Peanut Research Laboratory, USDA, ARS, Dawson, GA.

Ascomycetous fungi of the genus *Aspergillus* comprise a wide variety of species of biotechnological importance (e.g. *A. sojae*, *A. oryzae*, *A. niger*) as well as pathogens and toxin producers (e.g. *A. flavus*, *A. parasiticus*, *A. fumigatus*, *A. nidulans*). With the exception of *A. nidulans*, which is a homothallic fungus, these species were previously thought to be strictly asexual. Recent studies report *A. fumigatus* to be heterothallic and possibly undergoing sexual reproduction. Our population genetic analysis of the aflatoxin gene cluster in a Georgia population of *A. parasiticus* showed evidence of four distinct recombination blocks each showing a pattern of significant linkage disequilibrium and shared evolutionary history, thus providing indirect evidence of the possibility of sexual recombination in nature. We therefore investigated whether compatible mating type "MAT" genes (*MAT1-1* alpha box gene and *MAT1-2* HMG gene) were present. We found that each distinct *A. parasiticus* chemotype lineage (OMST and G_1 dominant) was heterothallic and possessed a single MAT locus, containing either a *MAT1-1* (G_1 dominant) or a *MAT1-2* (OMST) idiomorph. We observed a similar heterothallic organization of the MAT locus in *A. flavus* sampled from the same Georgia field but there was no association of mating type idiomorph with a specific chemotype lineage. The existence of both mating type idiomorphs in equal proportions in *A. parasiticus* and *A. flavus* populations indicates the potential for a cryptic sexual state in these agriculturally important species.

104. **What makes a pathogenic fungus? A comparative genomic analysis of *Coccidiodies* reveals the evolutionary rise of pathogenicity.** Thomas J. Sharpton, Jason E. Stajich and John W. Taylor. Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA email: sharpton@berkeley.edu

Knowledge regarding the evolutionary origins of pathogenicity is currently limited, but ultimately necessary to tackle disease. We employed evolutionary comparative genomic techniques to evaluate the origins of the pathogenic phenotype of *Coccidioides*, a Eurotiomycete capable of causing disease in healthy human adults. Comparisons of the pathogenic genomes of *C. immitis*, *C. posadasii* and the close, non-pathogenic relative *Uncinocarpus reesei* revealed genes unique to the pathogens and genes rapidly evolving under selective pressure, perhaps to escape the host immune system. This analysis reveals putative antigens and pathogenicity genes that will be investigated further in vivo. Additionally, surveys of gene family size across the fungal kingdom reveal unique characteristic expansions and contractions of certain families in the Onygenales, which contains several dimorphic pathogens including *Coccidoides*. Strikingly, families that experience significant reduction in the dimorphic pathogens appear to play a role in plant pathogenicity. Additionally, *Coccidioides* appears to have undergone expansions in gene families known to contribute to pathogenicity in *Coccidioides* as well as non-Onygenalen pathogens. This analysis should serve to identify possible drug and vaccine targets for *Coccidioides* and ultimately clarify the origins of the genetic architecture that enables a pathogenic phenotype in the Onygenales.

105. **Evidence of sexual reproduction in the Septoria speckled leaf blotch pathogen, *Septoria passerinii*, in the upper Great Plains.** Seonghee Lee and Stephen M. Neate. Department of Plant Pathology, North Dakota State University, Fargo 58105- 5012, USA. Email: sul22@psu.edu

Fifty seven polymorphic AFLP markers and mating type idiomorphs of *Septoria passerinii* were used to examine the sexual reproduction of the barley pathogen under field conditions. A total of 496 *Septoria passerinii* isolates were collected from 19 widely distributed barley fields in North Dakota and western Minnesota. The level of genetic and genotypic diversity was low within a lesion, but the higher level was found within a leaf and field. Population differentiation was low among field populations, and a significant correlation between geographical distance and genetic distance was not found. In addition, two mating types of *Septoria passerinii*, *MAT*-1 and *MAT*-2, were frequently found in isolates taken from individual leaves. The chi-square test revealed that equal frequencies of both mating types were observed in a leaf and field population. Moreover, when the data for all 19 sites was pooled, the overall ratios for mating types did not statistically differ from 1:1 ratio. The equal frequencies of both mating types are consistent with a sexually reproducing fungal population. Thus, we conclude that sexual recombination is occurring in *S. passerinii* under field conditions in the upper Great Plains.

106. **Comparative functional genomics: Reconstructing the evolution of central carbon metabolism in 13 fungal species**. Dawn Thompson[1] and Aviv Regev[1,2]. [1]Broad Institute of MIT and Harvard and [2]Department of Biology, MIT, Cambridge, MA USA

Molecular networks are the information processing devices of cells and organisms, transforming extra- and intra-cellular signals into coherent cellular responses. Networks are also remarkably flexible and can re-configure in an adaptive response to perturbation. We seek to understand the mechanisms by which molecular networks accommodate changes by analyzing genomics data through the unifying abstraction of the functional module. Specifically, we focus on evolutionary changes that occur in the organization and regulation of central carbon metabolism in *Ascomycota* fungi. This is the cornerstone metabolic module of yeast cells. In free-living *Ascomycete* yeast species the constantly changing environment dictates that nutrient availability is the major factor controlling growth and development. To survive in a highly competitive ecosystem yeasts have evolved to take up and metabolize a variety of carbohydrates, the most important of which is glucose. In the model organism *Saccharomyces cerevisiae* the central carbon metabolic network has been clarified in great detail, but the regulatory network is less well characterized. Although central carbon metabolism follows the same general theme in all yeasts, important biochemical, genetic and regulatory variations exist. For example, physiologically yeasts can be classified into respiro-fermentative and respiratory during aerobic growth on glucose. The former is characterized by high glucose uptake and ethanol secretion rates, and low biomass yield (*Saccharomyces cerevisiae* and close relatives) and the latter by low glucose uptakes and high biomass yield (e.g. *Kluyveromyces*). In addition, for species other than *Saccharomyces cerevisiae*, many of which have important industrial uses and some are human pathogens, basic knowledge of this and other fundamental pathways is limited. We are setting up a genomics platform to study a set of 13 fully sequenced fungal species spanning over 300 million years of evolution. We grow each organism under a variety of steady state conditions (e.g. rich glucose, alternative carbon sources) and along transitions from one condition to another. We will then measure a variety of phenotypes, most notably growth characteristics and genome wide expression profiles (followed by metabolomics and proteomics). We will use our novel computational algorithms for orthology mapping (SYNERGY) and regulatory network reconstruction (CISPROF), along with novel algorithmic development, to reconstruct the regulatory modules in each extant species, and the modules that existed in ancestral (extinct) ones. We will then mine those reconstructions for general patterns of how module content and regulation evolves and how changes occur at different molecular levels

107. **The course of evolution of *Magnaporthe oryzae Eleusine* pathotype inferred from phylogenetic trees and structures of the flanking region of avirulence gene *PWL1*.** Masaki Tanaka, Hitoshi Nakayashiki, Yukio Tosa, Shigeyuki Mayama. Faculty of Agriculture, Kobe University, Hyogo, Japan.

The blast fungus *Magnaporthe oryzae* is composed of host-specific subgroups. We examined the population structure of a subgroup, *Eleusine* isolates, using DNA makers (MGR586, rDNA-ITS, and *grasshopper*). The population of *Eleusine* isolates was divided into two groups, A and B. Interestingly, this grouping corresponded to the distribution of avirulence gene *PWL1*; all the B group isolates carried *PWL1* whereas the A-group isolates did not. Furthermore, the presence/absence of *PWL1* in *Eleusine* isolates was correlated with their pathogenicity toward weeping lovegrass (*Eragrostis curvula*); the A group, non-carrier of *PWL1*, was pathogenic on *Eragrostis* species. We assumed that the A group may be derived from an *Eragrostis* pathogen. To test this hypothesis, we constructed phylogenetic trees of *M. oryzae* isolates using rDNA-ITS, NUT1, Mdim2, beta-tubulin, Calmodulin, and EF-1alpha genes. In a combined tree the A group was clustered with *Eragrostis* isolates, not with the B group., as expected. Then, we cloned flanking regions of *PWL1* in the A group, the B group, and *Eragrostis* isolates, and compared their structures. The A group and *Eragorstis* isolates carried a gene for a hypothetical protein (Acc. XM_369238) in a region corresponding to *PWL1*. In the B group isolates, however, most of the DNA fragment for the hypothetical protein was substituted with *PWL1*. These results suggest that the B group may have acquired *PWL1* by horizontal transfer or recombination. The two-groups in the *Eleusine* isolates seem to have evolved through different courses.

108. Homing in on sexual recombination and spore production in the *Cryptococcus neoformans* species complex. Nathan Saul*^, Tien Bui†, Mark Krockenberger^, Richard Malik^ and Dee Carter†. *Molecular & Microbial Biosciences, and ^Faculty of Vet. Science, University of Sydney, NSW, Australia. d.carter@mmb.usyd.edu.au

Cryptococcosis, caused by *Cryptococcus neoformans* and *C. gattii* initiates with the inhalation of an infectious propagule. This is likely to be a basidiospore as yeast cells are encapsulated and unable reach lung alveoli. Spores can be produced sexually via the union of MATalpha and MATa cells, however most populations are overwhelmingly MATalpha. Recent studies have found recombinant spores can also be formed following MATalpha-MATalpha unions. Finally, some MATalpha strains can produce asexual spores, but there is little evidence of this in the genotypes responsible for most cryptococcal infections. A population genetics approach was used to investigate whether sexual recombination, and by implication the production of recombinant spores, had occurred in populations consisting of MATa and MATalpha cells, and in those that were MATalpha only. *C. gattii* isolates were obtained from a single *Eucalyptus camaldulensis* tree hollow that contained both mating types, and from a hollow in an area where only MATalpha cells occur. *C. neoformans* var. *grubii* isolates obtained from infected animals known to live exclusively in Sydney where MATa cells have never been reported, were also investigated. Sexual recombination was evident in all populations. Key to this finding were 1) selection of populations restricted in time and geographic space; and 2) preliminary phylogenetic analysis to identify recombining subpopulations within larger populations that initially appeared clonal.

109. **Genome structure impacts molecular evolution at the *AvrLm1* avirulence locus of *Leptosphaeria maculans.*** L. Gout[1,3], M.L. Kuhn[1], L. Vincenot[1], L. Cattolico[2], S. Bernard-Samain[2], A. D'Armaillé[1], M.-H. Balesdent[1], and T. Rouxel[1]. [1]INRA, PMDV, Route de St Cyr, 78016 Versailles, France. [2]Genoscope-centre national de séquençage, 2 rue Crémieux, 91057 Evry, France. [3]Protection des Plantes, INA-PG, 78850 Thiverval-Grignon, France

The Dothideomycete *Leptosphaeria maculans* is the most damaging disease of oilseed rape (*Brassica napus*) worldwide. In France, disease control relies mainly on the use of disease-resistant cultivars. The *Rlm* genes effectively control the disease as long as the corresponding avirulent allele (*AvrLm*) dominates in the pathogen population. However, *L. maculans* has the ability to rapidly adapt to the selection pressure exerted by a novel resistance gene as exemplified by the 3-year evolution towards virulence at the *AvrLm1* locus in French field conditions. *AvrLm1* was recently cloned and shown to be a solo gene within a 269-kb non-coding, heterochromatin-like region consisting of mosaics of degenerated repeats. We fully or partly sequenced the *AvrLm1* genomic region in one avirulent and two virulent isolates. The gain of virulence was linked in both cases with a 260 kb deletion of a chromosomal segment spanning *AvrLm1* and deletion breakpoints were identical or similar for both the virulent isolates. Among 343 field isolates analyzed, a similar large deletion leading to chromosome length polymorphism was evidenced by multilocus haplotype analysis in > 90% of the virulent isolates. Deletion breakpoints were strongly conserved in most of the virulent isolates leading to the hypothesis that a unique event of large deletion leading to the *avrLm1* virulence has diffused in populations or is strongly constrained by the genome environment. Surprisingly, even though a large chromosomal fragment was lost to gain virulence, no effect on fungal and pathogenic fitness could be evidenced, either in vitro or under natural field infection conditions.

110. **Intron evolution in basidiomycete laccase genes.** Sreedhar Kilaru [1], Pierre-Emmanuel Courty [2], Patrik J. Hoegger [3], Timothy Y. James [4], Francis Martin [2] and Ursula Kües [3]. [1] School of Biological Sciences, University of Bristol, Bristol, UK [2] Centre INRA de Nancy, Champenoux, France [3] Molecular Wood Biotechnology, Georg-August-University, Göttingen, Germany* [4] Department of Biology, Duke University, Durham, NC, USA

Most but not all homobasidiomycetes possess laccase genes. *Coprinopsis cinerea* was shown to contain 17 different laccase genes that could be divided into two gene subfamilies by intron positions. Here, we analyze a total of 835 introns in 74 different homobasidiomycete laccase genes related to the *C. cinerea* subfamily 1 laccase genes. These introns distribute over 75 different positions over the length of the genes. An intron phase-analysis shows a 2:1:1 distribution for phase-zero, phase-one and phase-two insertion within codons and thus a bias towards phase-zero. Two ancient introns occur in phase-zero position of histidine codons, regardless of the taxonomical order. Other ancient introns were lost from individual orders whilst new ones were apparently acquired at different time points during laccase gene evolution. Some of these other intron positions distinguish genes from Agaricales, Aphyllophorales and Ceratobasidiales. Within an order, there are also species- or genus-specific intron positions. The data suggest that particularly in the Agaricales, laccase genes duplicated several-fold after division into families, genera or species. A phylogenetic tree constructed by intron position conservation grouped laccase genes primarily according to taxonomy. In contrast, in a phylogenetic tree based on protein similarity, laccases clustered partially by the fungal life-style (white rot versus litter-decomposing species). *Work in our laboratory is supported by the DBU (Deutsche Bundesstiftung Umwelt).

111. **Missing links in *Mycosphaerella graminicola.*** Sarrah Ben M'Barek, Alexander H.J. Wittenberg, Theo A.J. van der Lee, Sarah B. Ware, Henk J. Schouten and Gert H.J. Kema Plant Research International B.V., P.O. Box 16, 6700 AA Wageningen, The Netherlands

Mycosphaerella graminicola (Fückel) J. Schröt. in Cohn is a haploid ascomycete that causes septoria tritici blotch in durum (*Triticum turgidum* L.) and in bread wheat (*T. aesitivum* L.). Two high-density genetic linkage maps with over 2000 Diversity Arrays Technology (DArT), AFLP and SSR markers were generated from two mapping populations derived from crosses between the sequenced isolate IPO323 and either isolate IPO94269 or IPO95052. Graphical genotyping revealed that some of the progenies lacked one or more linkage groups that were present in both parental isolates. PCR's with primers derived from the SSR and DArT markers positioned on these linkage groups were used to verify the absence of these linkage groups in off spring isolates. We confirmed the absence of six linkage groups in the IPO323 x IPO94269 and IPO323 x IPO95052 progenies. We could not conclusively confirm the absence of two additional linkage groups, maybe due to the presence of translocations. We studied the origin and effect of this phenomenon. Our results demonstrate that the chromosomes were lost during meiosis possibly by non disjunction. The loss of chromosomes did not seem to affect viability, pathogenicity or fertility as back crosses between these progeny isolates and the parents produced significant numbers of off-spring isolates. The finished genome sequence of isolate IPO323 and the aligned DArT markers from the high-density map enabled us to identify the missing chromosomes containing apparently redundant genes. Interestingly, we also identified isolates in the IPO323 x IPO94269 progeny that were diploid for one linkage group. We hope our data will contribute to a better understanding of the genetics in Mycosphaerella and redundancy of its genomic content.

112. **Simple sequence repeat (SSR) markers for *Pythium aphanidermatum, P. irregulare*, and *P. cryptoirregulare*.** Seonghee Lee and Gary W. Moorman. Department of Plant Pathology, The Pennsylvania State University, University Park, 16802, PA. USA. Email: sul22@psu.edu

Six microsatellite-enriched genome libraries from three *Pythium* species, *P. aphanidermatum*, *P. irregulare*, and *P. cryptoirregulare* were constructed to develop simple sequence repeat (SSR) markers. Four synthetic di-, $(AG)_{12}$, $(AC)_{12}$, $(GT)_{12}$, and $(CT)_{12}$, and three trinucleotides repeats, $(GGT)_6$, $(AAG)_8$, and $(AAC)_6$, were used to screen microsatellite loci in the three *Pythium* species. Approximately 600 positive recombinant clones for each *Pythium* species were selected and sequenced. About 35 % in *P. aphanidermatum*, 17 % in *P. irregulare*, and 25 % clone sequences in *P. cryptoirregulare* contained the unique simple sequence repeats (> 4 repeats). Total 110 SSR primer pairs for *P. aphanidermatum*, 73 SSRs for *P. cryptoirregulare*, and 82 SSRs for *P. irregulare* were developed and tested for amplifications and polymorphisms on four isolates of each *Pythium* species. Twenty three in *P. aphanidermatum*, 39 in *P. irregulare*, and 41 polymorphic SSRs in *P. cryptoirregulare* were found. After screening the polymorphic SSR markers to 8 isolates of each species, the most polymorphic and reproductive SSR markers were developed for each *Pythium* species; 9 in *P. aphanidermatum*, 18 in *P. irregulare*, and 23 in *P.cryptoirregulare*. These newly developed SSR markers can be useful for monitoring the introduced isolates and population genetic studies.

113. **Development of *Phlebiopsis gigantea* as a biocontrol agent for annosum root disease in the southeastern United States.** Brian Higgins and Sarah F. Covert. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA

The white-rot basidiomycete *Heterobasidion annosum* causes root and butt rot in conifers across temperate climate zones worldwide. In freshly thinned pine stands in the southeastern U.S., the ability of *H. annosum* to spread from cut stumps to live trees often results in significant volume loss. *Phlebiopsis gigantea*, another white- rot basidiomycete, has been used successfully across Europe to control *H. annosum*, but the U.S. Environmental Protection Agency (EPA) has not approved the use of *P. gigantea* in the U.S for this purpose. In order to develop a *P. gigantea* biocontrol product that will meet EPA regulatory approval, we have collected 64 isolates of *P. gigantea* from 14 sites in the southeastern U.S. Our early results suggest that *P. gigantea* isolates from the southeastern U.S. make sufficient spores in culture for commercial application of a spore suspension to cut stumps, and they are at least as effective in controlling *H. annosum* infection in southern pine wood as two commercially available *P. gigantea* isolates from Europe, known as RotStop (Finland) and PG1 (U.K.). We have developed 8 microsatellite markers to measure *P. gigantea* genetic diversity. These data will indicate how commercial application of selected *P. gigantea* strains is likely to impact local *P. gigantea* population structure. Current data on *P. gigantea* genetic diversity also be presented.

114. **Relationships between Alleles at Lineage Diagnostic Loci in *Fusarium graminearum*.** L.L. Anderson[1], Y-.W. Lee[2], R.L. Bowden[3], and J.F. Leslie[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS; [2]School of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul, Korea; [3]USDA-ARS Plant Science and Entomology Research Unit, Manhattan, KS.

DNA sequences of three nuclear genes (*MAT-1-1-3*, *TRI101* and *RED*) were examined from over 500 isolates of *Fusarium graminearum* collected from South American and Korean wheat, maize and sorghum. Genetic networks were developed for each gene that illustrate the relationships between the DNA sequences of isolates in this study based on the minimum number of base pair changes that separate the isolates. Some lineage diagnostic single nucleotide polymorphisms (SNPs) are not conserved in this strain set. The lack of dichotomous branching results in polytomies, which suggests that the lineages did not evolve in a stepwise fashion. This is further supported by the inconsistent location of lineages within the networks. For example, in the *MAT* network, Lineage 3 is in close proximity to the center of the network, whereas in *TRI101* it is one of the most distal. Additionally, several of the genetic networks cannot be resolved without cycles, which is consistent with recent gene flow between the lineages.

115. **Genetic dissection of *Neurospora crassa* circadian clock using two independent QTL mapping analyses.** Tae Sung Kim[1], Benjamin Logsdon[2], Jason Mezey[2], Kwangwon Lee[1]. [1]Dept of Plant Pathology, [2]Dept of Biological Statistics and Computational Biology, Cornell University, Ithaca NY 14853

Neurospora crassa has been a successful model organism in the circadian clock study for the past four decades. In an attempt to identify novel genetic components that are contributing the clock phenotypes, we relied on the quantitative genetics approach, Quantitative Trait loci (QTL) analysis. We constructed three independent mapping populations, whose parents are from geologically isolated locations. Two circadian clock phenotypes, period and phase, were evaluated in the 188 F1 progenies of each mapping population for the QTL analyses. Simple sequence-repeat- markers-based linkage maps that cover the whole genome with an average 13 cM interval were developed for the current study. To identify the clock QTLs, we performed two independent QTL mapping analyses, composite interval mapping (CIM) and Bayesian QTL mapping (BQM). For the two clock phenotypes, the CIM analysis identified 17 genetic loci from the three populations. BQM analysis confirmed all the 17 QTLs from CIM and furthermore detected additional 23 QTLs, suggesting that BQM has more power in identifying QTLs than CIM. 15 QTLs were co-localized with the previously identified clock genes, however, 25 of the identified QTLs were not co-localized

with any previously identified clock genes. Our findings demonstrate that QTL analysis is a powerful tool identifying novel genetic elements on complex phenotypes.

116. **Mites act in distribution of fungal spores in *Coprinopsis cinerea.*** Monica Navarro-Gonzalez [1], Wassana Chaisaena [1], Olivia Sanchez-Hernandez[1], Stefan Schütz[2] and Ursula Kües [1]. [1]Institute of Forest Botany, and [2]Institute of Forest Zoology and Forest Conservation, Georg- August-University, Göttingen, Germany

Mites are attracted to fungal cultures of *Coprinopsis cinerea* in order to graze on the mycelium. Both monokaryotic and dikaryotic mycelium are eaten by the mites including asexual spores (oidia). In vegetative cultures, only the melanized sclerotia are left, supporting a function in nature for duration: on fresh media, these sclerotia germinate. Upon fruiting body induction, primordia are also refused by the mites. When fruiting bodies matures, one can observe mites to climb up the stipe to feed on the autolysing cap tissues with the basidiospores. Stipes are refused as the sclerotia. Basidiospores are ingested but not digested. Faecal pellets are formed with around 400 basidiospores. These spores germinate and can easily form a dikaryon since the high number of eaten spores ensures that all four possible mating types arising from meiosis are represented. The fast moving mites thus contribute to the distribution of basidiospores in the environment and possibly to mixing populations in the nature. Mites lay eggs in close vicinity to spore pellets. This raises the idea that there might be a form of symbiotic interaction between mites and the fungus since germinated fungal colonies will serve as food for the hatching larvae. MNG, WC and OSH were funded by CONACYT (Mexico), the Rajamangala University of Technology and the DAAD.

117. **Adaptive evolution of the blast pathogen *Magnaporthe oryzae* populations on cereal hosts in Africa.** S. Sreenivasaprasad[1], J. P. Takan[1], S. Muthumeenakshi[1], J. Chipili[1], N. J. Talbot[2], E. O. Manyasa[3] and Y. Sere[4]. [1]Warwick HRI, University of Warwick, Warwickshire, CV35 9EF, UK. [2]University of Exeter, UK. [3]ICRISAT, Kenya. [4]ARC-WARDA, Benin.

Blast caused by *Magnaporthe oryzae* is a major production constraint to key cereals such as rice and finger millet in sub-Saharan Africa. We have used molecular, biological and pathological assays to characterise the pathogen populations on these hosts. In West Africa, where intensive rice cultivation is relatively recent (less than 500 years), blast pathogen populations showed typical lineage based structure established by MGR586 fingerprinting. Skewed distribution of the two mating types *MAT1-1* and *MAT1-2*, high female sterility and low fertility were observed. Pathogen isolates revealed clear differences in compatibility on rice differentials exhibiting R gene-type interactions. In contrast, on finger millet, domesticated in East Africa a few thousand years ago, more than 190 blast pathogen haplotypes were identified. The pathogen populations revealed continuous variation (lack of clonality) reflecting sexual recombination. These indigenous blast populations were genetically distinct to those in Asia, and showed a near equal distribution of the mating types and high fertility. All 70 isolates tested showed basic compatibility to a range of finger millet varieties suggesting polygenic quantitative interactions. Thus the *M. oryzae* populations on rice and finger millet reveal distinctive adaptive evolutionary patterns related to crop domestication and agricultural intensification in sub-Saharan Africa.

118. **Evolution of Mitogen-activated Protein (MAP) Kinase Cascade Components in Filamentous Fungi**. C. Greenwald and H. H. Wilkinson. Texas A&M University, Plant Pathology and Microbiology, College Station, TX

All filamentous fungi contain three paralogous MAP kinase signaling cascades within their genomes. Each cascade consists of three phosphorylating enzymes (MAPKKK, MAPKK, and MAPK), which act sequentially to transduce and amplify an environmental signal from a receptor protein to downstream genes that are directly involved in phenotypic responses. Mutations in fungal cascade components are well known for dramatic phenotypic consequences including effects on pathogenicity, mating, asexual development, abiotic stress response, etc. We predicted that since MAP kinase cascades are central to fungal development and life history there would be evidence for strong conservation of these proteins. We compared the gene family trees for MAP kinase pathway components from eight filamentous fungal genomes representing a diversity of filamentous fungi (*Neurospora crassa, Aspergillus nidulans, Fusarium graminearum, Magnaporthe grisea, Botrytis cinerea, Chaetomium globosum, Sclerotinia sclerotiorum, Aspergillus flavus*). The analysis has revealed a higher rate of synonymous vs. non-synonymous mutations in the cascade component genes, from which we inferred they have undergone a history of purifying selection. As one might expect, evolution of genes upstream (e.g. environmental sensors) and downstream (e.g. transcription factors) from these cascades do not mirror this degree of conservation, in fact, orthologous genes are seldom maintained in all eight species.

119. **Mating type in *Coccidioides* species**. M. Alejandra Mandel[1,3], Bridget M. Barker[1,3], Scott Kroken[1], John N. Galgiani[2,3,4], Steve. D. Rounsley[1,2] and Marc J. Orbach[1,2,3] [1]Department of Plant Sciences, [2]Bio5 Institute, [3]Valley Fever Center for Excellence, University of Arizona and [4]Southern Arizona Veterans Administration Health Care System, Tucson, AZ

Coccidioides immitis and *C. posadasii* are the causative agents of coccidioidomycosis, also known as Valley fever, a respiratory disease endemic to the desert southwestern US, along with parts of Mexico, and Central and South America. It is caused by

inhalation of asexual spores that undergo differentiation to initiate the parasitic phase in host lungs and, if not controlled, disseminates to other parts of the body. *Coccidioides* is only known to propagate asexually by segmentation of hyphae to produce arthroconidia, although details of its growth in the environment are limited. However, each species of *Coccidioides* has a recombined population structure, suggesting sexual reproduction. The presence of mating-type genes is necessary, although not sufficient, for fungal sexual reproduction. In order to investigate the potential for sexual reproduction in *Coccidioides* we analyzed genome sequences for potential mating-type loci and have identified idiomorphs in *Coccidioides* indicative of a typical heterothallic ascomycete. Data will be presented that define the MAT1-1 and MAT1-2 idiomorphs of both *C. immitis* and *C. posadasii*, and show that they are highly conserved between the two species. The idiomorphs in *Coccidioides* are the largest reported for an ascomycete; MAT1-1 is 8 kb and MAT1-2 is 9 kb in length. The MAT1-1 idiomorph contains four putative ORFs, and the MAT1-2 idiomorph has five putative ORFs. Analyses of ~400 isolates of *C. posadasii* and *C. immitis* revealed that they contain either a MAT1-1 or a MAT1-2 locus, and that they are distributed in a 1:1 ratio. A detailed analysis of the putative ORFs, their expression and a comparison of the MAT loci to those of related fungi will be presented.

120. Characterizing the mating-type locus of the graminicolous *Colletotrichum*: Patterns of sex, selection and host specialization. Jo Anne Crouch[1], Michael R. Thon[2], Michele Groenner-Penna[3], Bruce B. Clarke[1], Adlane Vilas-Boas[3], Lisa J. Vaillancourt[4] and Bradley I. Hillman[1]. [1]Rutgers University, New Brunswick, NJ; [2] Texas A&M University, College Station, TX; [3]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; [4] University of Kentucky, Lexington, KY.

While most filamentous ascomycetes have a one locus, two allele (idiomorph) mating system, extensive sampling of *Colletotrichum* species (teleomorph=*Glomerella*, order *Phyllacorales*) has identified only a single idiomorph (*MAT1-2*, with HMG box), regardless of reproductive lifestyle, i.e., heterothallic (self sterile) or homothallic (self fertile). Genetic analysis of *C. graminicola* has suggested the *Colletotrichum* mating system is governed by at least two unlinked loci. In order to explore its unique mating strategy, we have cloned and characterized the entire ~17-kb *MAT* locus from representatives of the falcate-spored, grass-inhabiting *Colletotrichum* group. First, using multi-locus phylogenetics (3,400-bp, 4 genes), we identified 11 unique species in a highly resolved tree topology that was almost entirely congruent with host plant association. Next, using a combination of transposon-mediated cosmid sequencing, cosmid chromosome walking and PCR amplification, we identified 6 genes at the *MAT* locus, including the *MAT1-2*, DNA lyase, and putative anaphase-promoting complex. Locus organization was identical in all 11 species, irrespective of homothallism (e.g. *C. falcatum*) or heterothallism (e.g. *C. graminicola*), with no evidence for a *MAT1-1* idiomorph. Comparison of the *MAT* locus with the distantly related species C. gloeosporioides, a cosmopolitan pathogen of dicots, showed that this region has been broadly conserved across evolutionary time, with all genes arranged in the same chromosomal orientation, although several differences in gene length and the number of introns/exons were detected. An examination of polymorphism, divergence and selection pressures on the locus are currently in progress and will be discussed.

121. **Evolution of an avirulence homolog (Avh) gene subfamily in *Phytophthora ramorum*, causal agent of sudden oak death**. Erica M. Goss, Caroline M. Press, and Niklaus J. Grunwald Horticultural Crops Research Laboratory, USDA ARS, Corvallis, OR gosse@science.oregonstate.edu

Pathogen effectors can serve a virulence function on behalf of the pathogen or trigger a rapid defense response in resistant hosts. Sequencing of the *Phytophthora ramorum* genome and subsequent analysis identified a diverse superfamily of approximately 350 genes that share two protein motifs (RXLR and dEER) with the four known effectors in plant pathogenic oomycetes. These have been termed Avh (avirulence homolog) genes. While as a whole the genes in this superfamily share modest sequence similarity, small groups of closely related genes can be identified. We have investigated the molecular evolution of one such group of seven Avh genes. Microarray data suggests that four of these genes are expressed in isolate Pr-102. We sequenced the full coding region (approximately 400 bp) and flanking noncoding regions of each gene in the three *P. ramorum* lineages. The number of polymorphic sites within *P. ramorum* genes ranges from 1 to 12, suggesting different evolutionary pressures among genes. Analysis indicates that these genes contain both amino acids under purifying selection (e.g. in the signal peptide and RXLR and dEER motifs) and under positive selection. We have also been able to obtain the sequence of homologous Avh genes in the sister taxa *P. hibernalis* and *P. lateralis*, allowing for examination of the evolution of these genes across species.

122. **Withdrawn**

123. **Evaluating genetic diversity of Fusarium proliferatum from orchids in Hawaii.** Cassandra Swett[1], Laurel Anderson[2], John F. Leslie[2], and Janice Y. Uchida[1]. [1]Department of Plant and Environmental Protection Sciences, University of Hawaii, Manoa, HI, USA. [2]Department of Plant Pathology, Kansas State University, KS, USA.

Fusarium proliferatum is a recently introduced pathogen to the Hawaiian Islands, where it causes disease on orchids in commercial nurseries. The pathogen origin is unknown, although south-east Asia has been suggested as a source. In this study, we evaluated the genetic diversity of *F. proliferatum* haplotype populations from diseased orchids in three nurseries, one on the Island of Oahu, and two on Hawaii. Amplified fragment length polymorphisms (AFLP's) were generated for between 29 and 49

single spore isolates per nursery, using three primer sets. Total genetic diversity of *F. proliferatum* is described for the state of Hawaii, and haplotype population diversity compared within and among nurseries. This work can be used to monitor the *F. proliferatum* strain populations, to evaluate if introduction is still occurring, and for future comparisons to strains populations from potential sources, to establish pathogen origins.

124. **Implications of repeat-induced point mutated transposons during the evolution of *Colletotrichum cereale***. Jo Anne Crouch, Bruce B. Clarke and Bradley I. Hillman. Rutgers University, New Brunswick, NJ

The evolution of eukaryotic genomes can be greatly influenced by the activities of transposable elements; conversely, transposons can serve as valuable tools that may increase our understanding of genome evolution and diversification. Here, we report the discovery of five transposon species from the two major lineages (clades A and B) of *Colletotrichum cereale*: the DNA transposons *Collect1* and *Collect2* (*pogo* and *Tc1/mariner* families, respectively), the long terminal repeat (LTR) retrotransposons *Ccret1* and *Ccret2* (*Pseudoviridae* and *Metaviridae*) and the non-LTR retrotransposon *Ccret3*. Using computational tools, our data show that 21 of 35 unique transposon loci display the signature pattern of recurring rounds of repeat-induced point mutation (RIP), a genome defense mechanism unique to filamentous fungi that mutates duplicated sequences by inducing GC-to-AT transitions. Analysis of over 100-kb of sequence data showed the RIPped transposons possess a skewed A+T base composition (genome=51%; RIPped=68%), increased incidence of TA, CT and AG dinucleotides, and decreased representation of the dinucleotide pairs TG, CA, CG, GA and TC. RIP index values were also consistent with those observed for RIPped transposons from *Neurospora*, *Fusarium*, *Podospora* and *Aspergillus* species, with CA, CG and TG the most common context for GC-to-AT transitions. Although our data supported the identification of RIP mutation acting only on transposons in the *C. cereale* clade B genome, none of the clade A transposon sequences displayed any evidence of RIP activity. Nevertheless, identification of a RIPped *Ccret2* element in the closely related species *C. falcatum* suggests that RIP mutation predated cladogenesis between these taxa.

125. **Patterns of recombination and population differentiation among *Colletotrichum cereale* isolates from 27 host plant genera.** Jo Anne Crouch, Bruce B. Clarke and Bradley I. Hillman. Rutgers University, New Brunswick, NJ

In populations of the turfgrass anthracnose fungus *Colletotrichum cereale*, the prevailing mode of reproduction is thought to occur clonally, as inferred primarily through the lack of a teleomorph. Coupled with the visual absence of a sexual state, previous reports of clonal genotypes from RAPD and isozyme analyses contribute to the notion that *C. cereale* has endured throughout history as an asexual organism. Because a pathogen's mode of reproduction strongly influences the course of its evolution and the alternative hypothesis has never been adequately tested, we are evaluating patterns of variation in this species. In the present study, we used an extensive nucleotide sequence dataset (4 genes, 3,400 nucleotides) to examine a large, worldwide sampling of pathogenic isolates of *C. cereale* from turfgrass and their counterparts isolated from cereal crops (wheat, oats, barley) and prairie grasses. This dataset illustrated that *C. cereale* is subdivided into several lineages, each composed of primarily (but not exclusively) either turfgrass or non- turfgrass derived isolates. Next, RFLP analysis of three transposon species provided evidence for recombination by *C. cereale*, even in relatively small populations. Furthermore, 21 of 35 unique transposon loci, when evaluated for dinucleotide compositional bias, displayed the signature pattern of repeat-induced point mutation (RIP), a genome defense mechanism that functions only during meiosis. Taken together, our data rejects the presumption of clonality for *C. cereale* and, given the severe losses sustained in turfgrass systems due to this pathogen, strongly emphasizes the need for additional inquiry into the biology, mating and dispersal mechanisms of *C. cereale*.

126. **Examining diversity in populations of *Anisogramma anomala.*** Sara N. Baxer, Jo Anne Crouch, C. Reed Funk, Thomas J. Molnar, Bradley I. Hillman. Department of Plant Biology, Rutgers University, New Brunswick, NJ

While *Anisogramma anomala (Gnomoniaceae)* is a relatively harmless inhabitant of the native American hazelnut (*Corylus americana*), this fungus is a destructive pathogen of the introduced, commercially important European hazelnut (*C. avellana*). Using systematic greenhouse and field inoculations, we have shown the breakdown of several sources that have provided long-term resistance to *A. anomala* in the Pacific Northwest. These findings raise questions about the possibility of geographically isolated populations of the fungus and whether variability in the fungus could be responsible for the breakdown of resistance. To address these questions, we have collected and are evaluating 185 isolates of *A. anomala* from 37 sites across North America. Our initial survey by sequence analysis at 4 gene regions (ITS, EF-1alpha, BT2, CAL; 1865 nucleotides) demonstrated an extremely low level of genetic variation between the isolates, despite the fact that *A. anomala* is only known to reproduce sexually. Using a hybrid-capture protocol, we are currently assembling a genomic DNA library enriched for microsatellite marker sequences to examine diversity in *A. anomala* populations at high levels of resolution.

127. **Modern arbuscular mycorrhizal fungi are clonal: evidence based on population structure of *Glomus etunicatum*.** Henk C. den Bakker[1], Joseph B. Morton[2], Teresa E. Pawlowska[1]. [1]Department of Plant Pathology, 334 Plant Science Building, Cornell University, Ithaca, NY 14853-5904; [2] Division of Plant and Soil Sciences, 1090 Agricultural Sciences Building, West Virginia University, Morgantown, WV 26506-6108

Arbuscular mycorrhizal fungi (phylum Glomeromycota) are generally believed to represent a group of ancient asexual organisms. To test the hypothesis of clonality, we explored the population structure of globally distributed isolates of *Glomus etunicatum* with a particular focus on two intensely sampled populations from California, USA. We used 9 anonymous markers, two protein coding loci (Beta tubuline and GAPDH) and ribosomal markers to (1) infer the phylogenetic relationship of *G. etunicatum* and its closest relatives, (2) to detect recombination and (3) to infer global and local haplotype distribution. (1) Phylogenetic analyses showed that *G. etunicatum* represents a monophyletic group, that is nested within an undescribed species *Glomus* 'yellow'. (2) Gene trees inferred from the individual markers exhibited phylogenetic concordance. Based on this phylogenetic concordance and the outcome of various tests of recombination we have to conclude that *G. etunicatum* reproduces in a clonal fashion. Our population genetic study revealed 11 distinct haplotypes among the globally distributed isolates of *G. etunicatum*. Eight of these haplotypes were found in tropical and subtropical regions, one haplotype was only found in the Californian populations, while two haplotypes were found to occur worldwide, without any distinct geographic pattern. We hypothesize that these haplotypes may represent two clonal lineages that have dispersed rapidly all over the world due to human agricultural activities.

128. Microsatellite analysis of environmental isolates of the human pathogenic fungus *Coccidioides.* Bridget M. Barker[1,2], Scott Kroken[2], Marc J. Orbach[2]. [1]Graduate Interdisciplinary Program in Genetics, University of Arizona. [2]Department of Plant Sciences, Division of Plant Pathology and Microbiology, University of Arizona, Tucson, Arizona

Coccidioides consists of two species: *C. immitis*, proposed to be restricted to California, and *C. posadasii* in Arizona, Texas, Mexico, and Latin America. Their geographical separation is putative, as some Californian patients were infected with C. posadasii and some non-Californian patients were infected with *C. immitis*. Geographical separation has also been observed at the population level within each species. Atypical infections may be due to patients traveling to and being exposed in other regions than where they were diagnosed, or due to long distance wind dispersal of infective arthroconidia. Alternatively, the ranges of the two species and their constituent populations may overlap. To test these alternative hypotheses, we determined the genotypes of 65 isolates obtained from soil samples in Tucson, Arizona, and genotypes of isolates from domesticated and wild animals from Tucson. These new genotypes were added to the database of all previously characterized isolates, and submitted to phylogenetic and population genetic analyses. Without exception, soil and animal isolates from Tucson are *C. posadasii*. Additionally, we have not yet observed any obvious sub-structuring among isolates that infect humans, domesticated and wild animals, and those found in the soil. These Tucson isolates encompass a significant amount of the genetic diversity of *C. posadasii*, suggesting that gene flow is sufficient to maintain high diversity within the Tucson subpopulation. There also appears to be greater gene flow than previously observed between Arizona and adjacent Mexico, suggesting that the strongest population structuring of *C. posadasii* may occur between the Sonoran and Chihuahuan Deserts.

129. **Evolutionary genomics of the ectomycorrhizal fungus *Paxillus involutus***. Anders Tunlid, Tomas Johansson, Antoine LeQuere, Kasper Astrup Eriksen, Andres Schutzendubeln, Balaji Rajashekar, Björn Canbäck, Jenny Hedh, Peter Samson, Susanne Erland Department of Microbial Ecology, Lund University, SE 223 62 Lund, Sweden (anders.tunlid@mbioekol.lu.se)

It is well known that ectomycorrhizal (ECM) fungi can differ markedly in their ability to form mycorrhizae and to promote the growth of the host plant. Generally such phenotypic differences could be the result of variations in gene content, quantitative differences in gene expression, and structural differences in gene products. We have uced cDNA microarrays to compare the trancriptome and genomes of strains of *Paxillus involutus* . The analyses included Nau, that is not compatible with birch and poplar, and the two compatible strains Maj and ATCC200175. The array contained reporters for 1075 putative unique genes in *P. involutus* , derived from a collection of expressed sequence tags (ESTs). On genomic level, Nau and Maj were very similar. Only 16 out of 1,075 genes analyzed by microarray-based hybridizations had signals indicating differences in gene copy numbers. 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 showed that two of them have evolved at an enhanced rate in Nau due to relaxed or positive selection.

130. **Phylogenetic markers for the Genus *Aspergillus* developed from complete genome sequences**. S.M. Witiak[1], R.A. Samson[2], J. Varga[2], A. Rokas[3] and D.M. Geiser[1]. [1]Department of Plant Pathology, Penn State University, University Park, PA 16802 USA, [2]Centraalbureau voor Schimmelcutures, 3508 AD Utrecht, NETHERLANDS, [3] Broad Institute, 7 Cambridge Center, Cambridge MA 02142 USA. email: dgeiser@psu.edu

The genus *Aspergillus* comprises tremendous biological and ecological diversity, and comparative genomic research in the genus would be aided greatly by a detailed multilocus phylogeny. Complete genome sequences of eight *Aspergillus* species were used as a guide to identify gene regions suitable for multilocus phylogenetic analysis of the genus. Amino acid sequences identified as conserved among animal and fungal species were used to design PCR primers, based on DNA sequences aligned among the *Aspergillus* genomes. Primers were then screened among seventeen or more *Aspergillus* species representative of the known phylogenetic breadth of the genus, as well as *Penicillium* outgroups, to determine the breadth of their utility. To date, ten new loci have been amplified and/or sequenced successfully. These loci will be used to infer phylogenetic relationships at the genus

level, as well as among closely related species within sections and subgenera. Their utility as markers for multilocus sequence typing (MLST) and for inference of species boundaries will also be assessed.

Genomics and Proteomics

131. **Comparative genomics of *Candida glabrata* clinical strains and closely related species.** Cécile Fairhead[1]*, Christiane Bouchier[2], Christophe Hennequin[3], Laurence Ma[2], Héloïse Muller[1] and Bernard Dujon[1] 1 and 2: Génétique Moléculaire des Levures and Plate-Forme Génomique, Institut Pasteur, 25 rue du Dr Roux 75015 Paris, France 3: Parasitologie-Mycologie, Faculté de Médecine St-Antoine, 27 rue Chaligny 75012 Paris, France *cfair@pasteur.fr

The hemiascomycete *Candida glabrata* is currently the second agent of candidiasis in humans after *C. albicans*. The genome of *C. glabrata* strain CBS138 was released in 2004. This species is in fact closely related to *Saccharomyces cerevisiae* and they share the same ancestor which has undergone a whole genome duplication. Although it has apparently retained the genes of the mating pathway; *C. glabrata* is only isolated as a haploid and no mating has been observed in this species. We have studied several isolates of *C. glabrata* by microsatellite analysis, and also by obtaining and examining Random Sequence Tags from three of them. The same strains were also examined for several features, such as electrophoretic karyotype and mating type. We will present evidence for the mainly clonal reproduction of this species, and discuss the mechanisms that lead to chromosome length polymorphism. We have also produced and examined Random Sequence Tags from three species that are described as the closest to C. glabrata: *K. delphensis*, *C. castellii* and *K. bacillisporus*. We will report on the divergence between these species and evolution of their gene functions and genomes.

132. **Genome evolution in hemiascomycetous yeasts.** Claude Gaillardin[1] and The Génolevures Consortium[2]. [1]Microbiologie et Génétique Moléculaire, INA-PG INRA CNRS, Thiverval-Grignon, 78500, FRANCE. [2]Génoscope (Evry), Institut Pasteur (Paris), U. Strasbourg, U. Orsay, CNRS (Bordeaux), CEA (Saclay), IBMC (Strasbourg), Laboratoire de Chimie Bactérienne (Marseille), U. Louvain (Louvain-la-Neuve), INA-PG (Thiverval-Grignon). claude.gaillardin@grignon.inra.fr

Within the Génolevures project, we have initiated a large scale genomic survey of the entire phylum of Hemiascomycetes. Genetic maps were found to be poorly conserved. Rates and types of genome rarrangements appeared conserved between lineages but highly variable between lineages. This suggested that distinct evolutionary mechanisms operated in each phylogenetic branch within this otherwise homogeneous phylum. To confirm these findings, we choose to populate clades that were currently less scrutinized than the Saccharomyces or the Candida albicans clades. We focussed on the Kluyveromyces clade, which contains several species of applied interest. We compared the genomes of *K. thermotolerans* and *Zygosaccharomyces rouxii* to those of *K. lactis*, *K. waltii* and *Eremothecium gossyppii*. The proximity of these genomes, the absence of recent whole genome duplication and the overall conservation of large syntenic blocks facilitated whole genome alignments thus permitting robust annotation. Phylogenetic relationships between these species were revised using conserved othologues. Clusters of orthologous proteins were individually examined for possible cases of accelerated evolution, showing that species with high or low gene evolution rates coexist in the same clade.

133. **Analysis of the fumitremorgin gene cluster in a human pathogen, *Aspergillus fumigatus*.** Shubha Maiya[1], Alexander Grundmann[2], Kosalec I [3], Shu-Ming Li[2] and Geoffrey Turner[1]. [1]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 3QX, UK; [2]Eberhard- Karls-Universität Tübingen, Pharmazeutische Biologie, Auf der Morgenstelle 8, 72076 Tübingen, Germany. [3] Department of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb g.turner@shef.ac.uk.

Aspergillus fumigatus strains have been reported to produce a variety of secondary metabolites, including toxic prenylated alkaloids such as fumitremorgin C,and many putative secondary metabolic gene clusters have been identified in the genome reference strain Af293 following the completion of the genome sequence.A gene ftmA encoding a dimodular non-ribosomal peptide syntetase (NRPS) was found within one of the putative secondary metabolic gene clusters. ftmA was overexpressed in strain Af293 by insertion of multiple copies,and in the naïve host *Aspergillus nidulans*, which lacks the equivalent gene cluster,under the control of the *alcA* promoter.Though neither fumitremorgins nor the dipeptide intermediate brevianamide F,cyclo-L-Trp- L-Pro,could be detected in wild-type strains,brevianamide F accumulated in liquid cultures of both species following increased expression of the NRPS gene.The cyclic dipeptide brevianamide F is the precursor of a variety of prenylated alkaloids,including fumitremorgins A,B,C,tryprostatin B and verruculogen.RT-PCR indicated that some of the genes in this cluster are poorly transcribed in Af293.We have also observed that overexpressing both *ftmA* and *ftmB*,a prenyl transferase,under a regulatable promoter *alcA* in *A. fumigatus* and in *A.nidulans* led to the accumulation of tryprostatin B which is the next intermediate in the fumitremorgin pathway.In order to determine the function of other genes in the cluster and to understand why it is poorly expressed in Af293,we have begun analysis of a fumitremorgin producing strain Af6460.

134. **Transposons in biotechnologically relevant strains of *Aspergillus niger* and *Penicillium chrysogenum***. Ilka Braumann[1], Marco van den Berg[2] & Frank Kempken[1]. [1]Abteilung für Botanische Genetik und Molekularbiologie, Botanisches Institut und Botanischer Garten, Christian-Albrechts-Universität zu Kiel, Olshausenstraße 40, 24098 Kiel, Germany [2]DSM Anti-Infectives, Alexander Fleminglaan 1, 2613 AX, Delft, The Netherlands

Despite of the relatively late discovery of fungal transposons and despite of the small fraction of repetitive DNA in fungal genomes (1.4%, 2.9%, and 3% in the genomes of *Aspergillus oryzae*, *A. fumigatus* and *A. nidulans* (Galagan et al., 2005)), today virtually all types of eukaryotic transposons have been identified in fungi (reviewed in Pöggeler and Kempken 2004). However, the impact of transposons on their fungal hosts' genomic development is still a matter of debate. Transposition might play a role in enhancing recombination in asexual fungi (Rep et al., 2006). However, it often failed to identify actively transposing mobile elements in the genomes of fungal laboratory strains, possibly because of ongoing selection for phenotypic stability (Daboussi and Capy, 2003) . It is particularly interesting that relatively little is known about the role transposons have in industrial producing strains. We set out to investigate the transposon content in the genomes of biotechnologically important strains of the two fungi *Aspergillus niger* and *Penicillium chrysogenum* using the annotated genome sequences of strains CBS 513.88 and ATCC 28089. A compilation of transposon-like sequences identified in the two genomes is given. Single sequence sets have been analysed in more detail: For example the *P. chrysogenum* class II element PeTraII was identified, to our knowledge the first transposon known in this fungus. We further compared the distribution of selected elements in a set of different fungal strains, all emanating from each other, to investigate transposon mobility in classical strain improvement programs. As an additional approach to test for transposon activity we performed transposon trap analysis for both fungi. Moreover we are currently developing a system which allows us to test the activity of putative transposable elements identified during database analysis. References: Daboussi MJ, Capy P (2003) Annu Rev Microbiol 57, 275-99; Galagan JE, Calvo SE, Cuomo C et al. (2005), Nature 438, 1105-15; Pöggeler S, Kempken F (2004) In: The Mycota II, Genetics and Biotechnology, 2nd edition (ed. Kück U). Springer Verlag, Heidelberg, New York, Tokyo; Rep M, van der Does HC, Cornelissen BJC (2005) Fungal Genet Biol 42, 546-553

135. **Horizontal gene transfer as the source of extra genes in *Aspergillus oryzae.* Nora Khaldi[*], Kenneth H. Wolfe.** Smurfit Institute of Genetics. University of Dublin Trinity College Dublin 2. Ireland.

Although horizontal gene transfer (HGT) is a major aspect of prokaryotic evolution and is becoming increasingly documented between prokaryotes and eukaryotes, very few cases of the HGT of nuclear genes from eukaryote to eukaryote have been documented so far. Several obstacles impede the unequivocal demonstration of HGT. The detection of foreign genes is facilitated if the recipient and donor genomes are from two different kingdoms, but if they are relatively closely related then observations suggestive of HGT may be equally consistent with alternative scenarios of gene duplication and gene loss. *Aspergillus oryzae* is a filamentous fungus that has a large gene complement compared to its annotated relatives. Here we test three alternative explanations for the existence of these extra genes in *Aspergillus oryzae*: recent gene duplication, whole genome duplication, and HGT. We show using gene location, evolutionary rate, and phylogeny based methods that HGT took place from the close sister subphylum *Sordariomycetes* into an ancestor of *Aspergillus oryzae*. Finally, we investigate the role of the transferred genes and show that transfer played a role in enriching Aspergillus oryzae's gene repertoire, specifically by expanding its hydrolytic enzyme gene complement.

136. **Characterisation of the *Trichoderma reesei* proteasome**. L. Kautto[1,2], J. Grinyer1, P. L. Bergquist[1,2,3] , V. S. J. Te'o[1,2], and K.M. H. Nevalainen[1,2] [1]Department of Chemistry and Biomolecular Sciences, Macquarie University, Sydney NSW 2109, Australia. [2]Macquarie University Biotechnology Research Institute, Macquarie University, Sydney NSW 2109, Australia. [3]Department of Molecular Medicine and Pathology, University of Auckland Medical School, Auckland, New Zealand

The filamentous fungus *Trichoderma reesei* is one of the most efficient eukaryotic cell factories available. Considering its extraordinary secretion capacity, between 40 – 100 grams L-1 of protein, this species can be characterised as "a professional" protein secretor. High-level secretion proposes a challenge for the cellular machinery responsible for protein quality control, which is mainly carried out by UPR and ERAD. Ubiquitin-proteasome pathway plays a key role in the post –translational regulation in eukaryotic cells. Proteins that are not folded correctly or not fully assembled are recognised in the early secretory pathway featuring a large (approximately 2.4 MDa) multicatalytic protease, the proteasome. We are especially interested in exploring the function of the proteasome in ERADication of misfolded proteins from the secretory pathway by using mutant forms of an endogenous efficiently secreted cellulase enzyme as experimental molecules. We have produced a 2D master map for the *T. reesei* 20S proteasome. From this reference map, 13 of the 14 20S proteasome subunits and many interactive proteins that were co-purified with the 20S proteasome have been identified. We have prepared a series of mutant forms of the main cellobiohydrolase enzyme CBHI to trace their secretion and presumed degradation in the proteasome. We are currently in a process of characterising *T. reesei* transformants expressing the mutant proteins.

137. **Systems Biology of the Biological Clock**. Dong, Wubei Xiaojia Tang, Yihai Yu, Roger Nilsen, James Griffith, Jonathan Arnold, and H.-Bernd Schuttler. University of Georgia, Athens, GA 30602. arnold@uga.edu.

A model-driven discovery process called computing life is used to guide a series of 3 microarray experiments. As much as 25% of the *N. crassa* transcriptome is found to be circadian. A clock mechanism involving the genes wc-1, wc-2, and frq appears to have many different kinds of outputs including genes whose products are in DNA metabolism, RNA metabolism, cell cycle, protein metabolism, transport, carbon metabolism, development, isoprenoid (i.e., including carotenoid) biosynthesis, development, and varied signaling processes. The known clock mechanism involving the FRQ oscillator appears to explain only about 43% of the genes, which are circadian and light-responsive. Only about 58% of the White-Collar Complex (WCC)-controlled genes appear to be circadian or light-responsive, implying other control functions for WCC. In each of three cycles of microarray experiments through the computing life paradigm microarray data support that wc-1 and wc-2 should be autoregulated by WCC. Among 11,000 genes in the *N. crassa* genome a total of 295 genes, including a large fraction of phosphatases/kinases, appear to be under the immediate control of the FRQ oscillator as validated by 3 independent microarray experiments.

138. **Withdrawn**

139. **Comparative genome hybridization to examine genome variability in Cryptococcus species**. GG Hu, I Liu, A Sham, JW Kronstad. Michael Smith Laboratories, The University of British Columbia, Vancouver, BC, Canada

The basidiomyceteous fungal pathogen *Cryptococcus neoformans* includes four serotypes, A, B, C, and D, based on antigenic differences in the polysaccharide capsule that is the major virulence factor. The A and D serotypes mainly infect immunocompromised individuals, while B and C are now classified as a separate species, *C. gattii*, which infects both immunocompromised and immunocompetent patients. Strains with hybrid serotypes (AD and AB) have also been identified from clinical and environmental sources. There are also two mating types (MAT alpha and MATa) in each serotype, although strains of the alpha mating type are more frequently isolated and only genomes for MAT alpha strains have been sequenced. Genome sequence information is now available for five Cryptococcus strains (serotype A strain H99, serotype D strains JEC21 and B3501, and serotype B strains R265 and WM276). In the present study, we used the complete genome sequences of H99, JEC21 and WM276 to obtain high-density oligonucleotide genomic microarrays for comparative genome hybridization (CGH) experiments. We first examined the genomes of the MATa strain NIH433 and the MATalpha strain NIH12 that were the original parents of a cross that generated the strain JEC21. The hybridization of these genomes to a JEC21 array allowed the mapping of putative recombination sites and a comparison of the MAT regions. We then examined the genomic differences between MATalpha and MATa strains in serotype A strains (H99, 125.91, BT63), and serotype B strains (WM276 and E566). The CGH data for the MATalpha and MATa regions allowed us to establish the relationship between the CGH signal log ratio and sequence identity, and to interpret additional differences in genome content between the strains. The differences for a set of these regions were confirmed by PCR and sequencing. We further examined genome content of the AD hybrid strains KW5, CDC228 and CDC304 using both H99 (serotype A) and JEC21 (serotype D) genomic arrays. Interestingly, the CGH data indicated that chromosome 1 of all three AD hybrid strains and chromosomes 6 and 7 of KW5 most likely originated only from a serotype A genome. In addition, chromosome 5 of CDC304 and chromosome 8 of KW5 appear to originate from a serotype D genome. These conclusions are supported by PCR-RFLP analysis of selected sites on these chromosomes. Taken together, the CGH data provide the first insight into the differences in whole genome content among MATalpha and MATa strains and AD hybrids.

140. **Transcriptome analysis of *Lentinula edodes* mature fruiting bodies by novel pyrosequencing**. W.W.Y. Chum, I.S.W. Kwok, T.C.H. Au, H.S. Kwan. The Chinese University of Hong Kong. Shatin, HKSAR

Developmental processes of basidiomycetes from vegetative mycelium to mature fruiting bodies for sporulation are dramatic morphological changes. We attempted to understand the biological mechanisms through transcriptome analysis of fruiting bodies of *Lentinula edodes* using high-throughput Genome Sequencer 20 System (454 Life Science). Combined cDNA from mature fruiting bodies before and during sporulation of *L. edodes* were sequenced to isolate a total of 7481 cDNA contigs. Each contig was generated from a number of reads of cDNA that represent the number of cDNA fragments aligned for the contigs. Numbers of reads/length may be proportional to gene expression levels of the contigs revealed by comparison with LongSAGE results of *L. edodes*. All contigs were homology searched by BLASTX. However, there is no genome data available for *L. edodes* studies, we developed a program to match the contigs from other databases followed by categorization with Gene Ontology (GO). Among a total 1202 annotated contigs, 909 contigs matched to sequences in database and their biological processes were classified by GO. Top 100 contigs in numbers of reads/length were analyzed and some of them may be related to mature fruiting body development or spore formation. About 50 contigs were novel. To our knowledge, this is the first study in sequencing fungi cDNA by using the GS20 for transcriptome analysis. The results greatly advance our knowledge in biological mechanisms of mature fruiting body and sporulation of mushroom.

141. **Transcriptome analysis reveals differentially expressed genes involved in increased nuclear kinetics and polar growth in the *Aspergillus nidulans* ATM (*atmA*) null mutant.** Iran Malavazi and Gustavo H. Goldman, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil

Ataxia telangiectasia (AT) is an inherited disorder characterized by progressive loss of motor function and susceptibility to cancer. Numerous studies have emphasized the role of the affected gene product, ATM, in the regulation of the DNA damage response. Recently, in addition to its expected role in the DNA damage response, we found that the *Aspergillus nidulans* homologue, AtmA, is also required for nuclear proliferation and polarized hyphal growth. For a comprehensive evaluation of genes that have their expression modulated by ATM, here by using a microarray hybridization approach, we performed two sets of comparisons of the mutant with the wild type strain: (i) growth in complete medium for 60, 90, and 120 minutes, and (ii) HU block-release experiments for polar growth (60, 90, and 120 minutes release after 5 hours HU-growth). The first set of experiments revealed that several genes involved in DNA replication and in the pentose phosphate pathway (ppp) are more expressed in the delta *atmA* mutant. We constructed double mutants for delta *atmA* and two mutations impaired in the ppp, *pppA* (probably encoding a transaldolase) and *pppB*. The double mutants have a decreased nuclear proliferation rate when compared to the delta atmA mutant, suggesting that the increased expression of the ppp genes could be important for increased nuclear proliferation in the delta atmA mutant. The second set of experiments with global expression associated to polar growth revealed a decreased mRNA expression for the following group of genes in the delta atmA mutant: (i) genes involved in the formation of a polarized hyphae (e.g., cdc42) and control of polar growth; (ii) genes involved in the synthesis of phosphatidic acid (1,2-diacyl-sn-glycerol 3-phosphate); and (iii) genes involved in the ergosterol biosynthesis (plasma membrane microdomains, lipid rafts). We are currently investigating some of these genes by constructing double mutants with them and delta atmA. Financial support: FAPESP and CNPq, Brazil

142. **Transcriptional profiling of cross pathway control in *Neurospora crassa*: Comparative analysis of the Gcn4p and CPC1 regulogs.** Chaoguang Tian, Takao Kasuga and N. Louise Glass. Plant and Microbial Biology Department, University of California, Berkeley, CA 94720

Identifying and characterizing transcriptional regulatory networks is an important task in elucidating gene function and how these regulatory networks evolve. In *Saccharomyces cerevisiae* one of the most intensely studied transcription factors is the bZIP transcription factor, Gcn4p. Gnc4p is essential for transcriptional response when *S. cerevisiae* experiences amino acid starvation. In the filamentous ascomycete fungus, *Neurospora crassa*, the ortholog of GCN4, called cross pathway control (*cpc-1*) is required for the ability of *N. crassa* to induce a number of amino acid biosynthetic genes upon response to amino acid starvation. In this study, we decipher the CPC1 regulog by transcriptional profiling wild-type and a *cpc-1* mutant using full genome *N. crassa* 70-mer oligonucleotide microarrays. We show that 443 genes are either direct or indirect CPC1 targets, including 67 amino acid biosynthetic genes, 16 tRNA synthetase genes and 13 vitamin-related genes. Comparison between the *N. crassa* CPC1 and *S. cerevisiae* Gcn4 regulogs showed that regulation of only 18 genes is conserved between these two species, and primarily include amino acid biosynthetic genes. Exploration of regulatory networks in *N. crassa* and *S. cerevisiae* sheds light on how gene interaction networks evolve.

143. Development and evaluation of an Affymetrix array for *Aspergillus flavus*. Andrea Dolezal[1], David Ryan Georgianna[1], Greg OBrian[1], Charles Woloshuk[2], Nancy Keller[3], Jiujiang Yu[4], Dahlia Nielsen[1], Gary Payne[1]. [1] North Carolina State University, Raleigh, NC. [2] Purdue University, West Lafayette, IN. [3] University of Wisconsin, Madison, WI. [4]USDA/ARS/SRRC, New Orleans, LA.

A multi-species Affymetrix GeneChip array was developed to study development, metabolism, and pathogenicity of *A. flavus*. This chip, based on the whole genome sequence of *A. flavus*, contains 13,000 *A. flavus* genes, 8,000 maize genes, and 25 human and mouse innate immune response genes as well as the fumonisin and trichothecene clusters from *Fusarium*. These arrays were used to monitor gene expression of *A. flavus* during aflatoxin biosynthesis in defined media and during infection of developing maize seeds. A parallel study comparing this array with a 5002 element cDNA array showed the same expression profile for the aflatoxin biosynthetic genes when *A. flavus* was grown in culture on defined media at conducive and non conducive temperatures for aflatoxin production. Gene expression was also monitored in *A. flavus* during the infection of field grown maize seeds. The profile of aflatoxin gene expression by *A. flavus* in infected maize kernels was similar to that observed for *A. flavus* grown in the lab under conductive temperatures for aflatoxin production. In addition, several genes encoding enzymes for the metabolism of complex carbohydrates and for transporters were also elevated during infection of maize seeds. Nonspecific hybridization across species has not been observed in any of our experiments using the Affymetrix GeneChip. These initial observations show that these multi-species arrays will be a powerful tool for studying the complex ecology and metabolism of *A. flavus*. This research was funded by USDA/NRI/CGP 2006-35604-16666.

144. **Gene expression analysis of the basidiomycete wood decay fungus (*Heterobasidion parviporum*) during adaptive growth on wood and toxic cell wall phenolic (ferulic acid)**. Fred O. Asiegbu[1,*], Guosheng Li[1] and Jason Osborne[2].

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The success of many wood rotting fungi lies in their ability to overcome and survive in a phenol-phytoalexin rich toxic environment within litter, wood debris and tissues. Although so much has been learned about the ecology, taxonomy and physiology of several wood decaying basidiomycete fungi, the molecular basis for their survival in a diverse range of substrates and ecological habitats has been very little studied. Using the necrotrophic wood decay fungus (*Heterobasidion* sp.) as an experimental model, we investigated its transcriptome response during growth on a woody substrate and further compared how the gene expression pattern is affected by exposure to toxic cell wall phenolics (ferulic acid), simple sugars (glucose) and oxalic acid (a component of hyphal exudates). We documented elevated transcription of significantly more genes in glucose starved media (such as in ferulic acid) which probably indicates that the phenolic compound may constitute an important alternative energy source during periods of glucose starvation. The result also revealed similarity in expression profiling pattern of oxalate and glucose media or ferulic acid and wood which suggest that the fungus might use the same genetic programs for responding to growth on certain substrates. The results also indicate that some genes in fungi that determine the outcome of growth on a diverse range of substrates may share common regulatory pattern. The importance of the results in the identification of genes controlling important traits and their regulation during exposure to different substrates as well as how it applies to other related wood rotting fungi is discussed.

145. **Comparative *Verticillium* Genomics.** Steven J. Klosterman[1] Krishna V. Subbarao[2], Katherine F. Dobinson[3], Paola Veronese[4], Seogchan Kang[5], Scott E. Gold[6] and Li-Jun Ma[7]. [1]USDA, Agricultural Research Service, Salinas, CA [2]University of California, Davis, CA [3]Agriculture and Agri-Food, London, ON Canada [4]North Carolina State University, Raleigh, NC [5]Penn State University, University Park, PA [6]University of Georgia, Athens, GA [7]The Broad Institute of MIT and Harvard, Cambridge, MA

The genus *Verticillium* contains several phytopathogenic species, the most important being *V. dahliae*, *V. albo-atrum*, and *V. longisporum*. The soil-borne habitat of these species, and their capacity to infect a variety of crops, make them chronic economic problems in crop production. Verticillium wilt causes billions of dollars in annual losses worldwide. A comparative genomic approach has been initiated to study the evolutionary processes and genetic mechanisms that underpin the pathogenic development of soilborne fungi among *Verticillium* spp. For this purpose, we will generate a 7X genome assembly of *V. dahliae* and a 4X genome assembly of *V. albo- atrum*, two verticillium species that have distinct phenotypic variation and host ranges. Both of these assemblies, coupled with gene annotation, will provide the tools for comparative analyses to improve annotation, identify SNPs and chromosome structure changes, and create sets of conserved and species-specific genes among these two soilborne pathogens. In addition, 0.5X Fosmid sequence reads of *V. longisporum* will enable the assessment of whether the diploidization of *V. longisporum* arose as a result of inter- or intraspecific hybridization events. The synergistic power of comparative genomics will speed *Verticillium* research toward a greater understanding of pathogenicity. This knowledge will enable new methods for improving disease detection and management.

146. **Comparative genomic analysis of *Aspergillus fumigatus* and sequenced close relatives.** William C. Nierman[1], Vinita Joardar[1], Rama Maiti[1], Paolo Amedeo[1], David Denning[2] Jennifer Wortman[1], and Natalie Fedorova[1]. [1]The Institute for Genomic Research, Rockville, MD, USA. [2]School of Medicine and Faculty of Life Sciences, The University of Manchester, Manchester, UK

Comparative genomic analyses of pathogenic *Aspergillus fumigatus*, and closely related species revealed a large pool of variable genes, which may ensure the rapid adaptation to a range of environments. These variable genes are structurally distinct from the rest of the genome and tend to function in secondary metabolite biosynthesis, transport, and detoxification of xenobiotics. Many are up-expressed in a mammalian host suggesting that they may be essential for survival in this environment. In contrast, all previously identified virulence-associated genes are well conserved in all sequenced aspergilli and seem to be evolving under strong purifying selection. The variable genes tend to cluster together in subtelomeric and intrasyntenic chromosomal blocks called accessory zones that share several characteristics with prokaryotic genomic islands. The origin of accessory genes in aspergilli does not appear to involve lateral gene transfer. This analysis also shows that the aspergilli have utilized several mechanisms to ensure a quick diversification and turnover of secondary metabolism gene clusters in the course of evolution.

147. **Transcriptome analysis of shiitake mushroom *Lentinula edodes* development using multiple approaches.** W.W.Y. Chum, I.S.W. Kwok, X.L. Bian, K.T.P. Ng, R.S.M. Shih, G.S.W. Leung, T.C.H. Au, and H.S. Kwan. The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR

Fruiting body development is an important area in mushroom biology and have been analyzed at the molecular level. We aim to analyze the transcriptome during development of Shiitake mushroom *Lentinula edodes*. First, we used RNA fingerprinting with arbitrarily primed polymerase chain reaction (RAP or Differential Display) to isolate genes differentially expressed. Over 100 genes were isolated and sequenced. Fifteen were studied further. Second, cDNA clones were randomly sequenced to generate

about 1000 Expressed Sequence Tags (ESTs). Differential expression of the ESTs were analyzed by dot-blot hybridization and cDNA microarray analysis. Third, Serial Analysis of Gene Expression (SAGE) and LongSAGE were used to determine the proportion of each mRNA among total transcripts in various growth stages. About 30,000 transcripts were counted from seven developmental stages. Expression profiles of the tags were annotated and clustered. About 800 tags matched to our cDNA sequence collection. Forth, over 13000 cDNA sequence contigs were obtained by high-throughput sequence-by-synthesis technology. These cDNA were annotated using programs we developed. Fifth, differential expression of selected genes were confirmed using Northern analysis and real-time RT-PCR. A few genes were localized in various tissues by *in situ* RNA-RNA hybridization. Gene expression profiles revealed by different approaches were compared and were generally consistent.

148. **Isolation and analysis of genes expressed in lignocellulose-grown *Lentinula edodes* using novel sequencing-by-synthesis approach.** Iris S.W. Kwok, Winnie W.Y. Chum, Tommy C.H. Au and H.S. Kwan. Department of Biology, The Chinese University of Hong Kong, Shatin, HKSAR, China

The edible shiitake mushroom *Lentinula edodes* degrades lignin and cellulose efficiently and completely, so it has been widely used in bioconversion and bioremediation. A cDNA pool prepared from mycelium grown on lignocellulose was sequenced using pico-titered plate based genome sequencer (Roche). 5894 cDNA contigs were obtained. Their sizes ranged from 100bp to 2 kb. More than 2600 contigs could be annotated by BLAST homology search, one third of the matches were highly significant (E-value $>10^{-5}$). Using Gene Ontology (GO), these contigs were categorized according to their biological functions and processes involved. We found genes encoding ligninolytic enzymes and cellulolytic enzymes, such as chloroperoxidase, laccase, a and b-glucosidases, and endo-1,3(4)-b-glucanase. We have also obtained genes encoding antioxidative thioredoxins, glutaredoxin, superoxide dismutase (SOD) and catalase. The expression levels of SOD and catalases in mycelia grown in lignocellulose and non-lignocellulose media were compared with quantitative RT-PCR. Surprisingly, their expressions are higher in non-lignocellulose medium. This indicates that growing in lignin, *L. edodes* is not encountering strong oxidative stress despite that free radicals are produced to degrade lignin. These findings provide new insights on the lignocellulytic system and also the antioxidative activity during lignin degradation of *L. edodes*.

149. **The *Neurospora crassa* e-Compendium: integrating genetic and genomic data**. A. Radford, Biological Sciences, The University of Leeds.

The e-Compendium is the logical development of earlier printed Compendia (Barratt et al (1954) Adv Gen 6: 1-93; Perkins et al (1982) Microbiol Rev 46: 426-570; Perkins et al, The Neurospora Compendium, Academic Press, 2001), integrating the genetic data accumulated over seven decades with the genome sequence and annotations of the last few years. It presents on line, in searchable form, data on over 2,100 genes structured into fields for gene symbol, name, linkage, product, phenotype, contig, sequence, reference, etc., with web links to sequence at Broad, bibliography at Pubmed, enzyme at Expasy, structure at PDB, pictures of morphological mutants at various locations, etc.. The search function permits keyword searches singly and in combination in different database fields. The e-Compendium also includes compiled chromosome maps based on both genetic and genomic data, and including over 1,700 sequenced genes. These include the products of the author's recent studies of novel genes for tRNA, rRNA, proteases, glycosyl hydrolases etc.. Monitoring software shows that the e-Compendium has been used by over 1,300 individual users in the past 12 months, over half of whom have used the site repeatedly.

150. Searching for the Rosetta stones in the multifunctional proteins of the Phytophthora genomes. Tom Wittenschlaeger [1], Ryan Austin[2], Nicholas Provart[2], Paul F Morris [1]. [1] Department of Biological Sciences, Bowling Green State University, Bowling Green OH 43403, [2]Cell and Systems Biology University of Toronto, Toronto ON M5S 3G5.

Eukaryotic genomes have in common a large number of multifunctional proteins. A global survey of the oomycete *Phytophthora sojae* genome, identified 274 novel multifunctional proteins using strict criteria that excluded multi- exonic gene models without EST support. These *P. sojae* proteins have significant BLAST hits to two or more different proteins. Such proteins have been posited as Rosetta stones, since their association in one genome has been used to infer the association of orthologous proteins in other genomes. In our analysis, we adopted the reciprocal smallest distance algorithm (Wall et al 2003) to identify potential orthologs in 34 sequenced genomes. Surprisingly, this approach identified only seven potential Rosetta stones, where each domain of a multifunctional protein had an ortholog in the same organism. A separate phylogenetic analysis has identified several examples where each half of a multifunctional protein, clusters in a node with homologs from separate kingdoms. The evolutionary history of oomycetes involved the endosymbiotic acquisition of a red algae, and subsequent transfer of nuclear and plastid genes to the host nucleus. We postulate that this endosymbiotic event (genome acquisition and recombination) has enabled the ancestral genome to develop metabolic and regulatory pathways that are distinct from those of the animal, fungal and plant genomes. Oomycete pathways that include genes from plant and animal ancestral genomes may have metabolic and regulatory efficiencies that are not present in other organisms. Our observations suggest that the evolutionary strategy of genome acquisition and recombination should also be assessed in other members of the Chromalveolates.

151. **Full-Length cDNA Sequences for Genome Annotation in *Phytophthora infestans.*** William Morgan[1], Joe Win[1], Liliana Cano[1], Michael C. Zody[2], Chad Nusbaum[2], Sophien Kamoun[1]. [1]Department of Plant Pathology. The Ohio State University, Ohio Agriculture Research and Development Center, Wooster, OH 44691, USA. [2]The Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA.

Phytophthora infestans is a devastating phytopathogenic oomycete that causes late blight on tomato and potato. Recent genome sequencing of *P. infestans* and other *Phytophthora* species resulted in vast amounts of sequence data providing opportunities to unlock the complex nature of pathogenesis. However, accurate annotation of *Phytophthora* genomes is a significant challenge. Full-length cDNA (FLcDNA) sequences are essential for the correct annotation of genomic sequences and for downstream functional analyses. We initiated three separate approaches to generate novel FLcDNA resources for *P. infestans*. First, bioinformatics analysis of 31592 *P. infestans* 5' ESTs identified 4943 putative FLcDNAs predicted to contain a complete open reading frame. Of these, we sequenced the inserts of 480 clones corresponding to unique sequences. Second, we constructed and sequenced normalized FLcDNA libraries using mRNA isolated from various developmental stages of *P. infestans* strain T30-4 to acquire additional FLcDNA sequences. Finally, we constructed new cDNA libraries that are adapted for high throughput sequencing of 5' mRNA ends using 454 sequencing. The accurate picture of *P. infestans* gene structure that emerges will help us develop more precise gene predictions algorithms. In addition, the FLcDNA sequences will allow us to gauge the degree of sequence conservation between *P. infestans* genes and those of other oomycetes, and identify patterns of gene conservation between *P. infestans* and various eukaryotes.

152. **Proteomic analyses of developmental stage specific proteins in *Aspergillus nidulans*.** Yun-Hee Park[1], Eun Hye Kang[1], Sujin Kim[1], Ja-Young Yun[1], Kwang- Yeop Jang[2], Keon-Sang Chae[2], Dong-Min Han[3], Suhn-Kee Chae[4], Pil Jae Maeng[1] and Hee-Moon Park[1]. [1]Dept. Microbiol., SBB, Chungnam National University, Daejeon; [2]Div. Biol. Sci., Chonbuk University, Chonju; [3]Div. Life Sci., Wonkwang University, Iksan; [4]Dept. Life Sci. & Technol., Paichai University, Daejeon, South Korea.

Aspergillus nidulans which has three different cycles, such as asexual, sexual and parasexual cycle, showed various morphological changes during vegetative and development. For a global analysis of developmental stage specific proteins, we investigated the changes in protein expression profiles during developmental stage. We decided the time points for harvesting cultures which revealed specific change of morphology, such as vegetative growth 9 and 14 hr, asexual development 9 and 18 hr, hypoxia condition 12 hr and sexual development 1, 6, and 24 hr. We extracted total proteins from each stage and performed two-dimensional polyacrylamide gel electrophoresis (2-DE). All 2-DEs were independent and repeating three-times for every stages. The PDQuest image analysis of silver stained 2D-gel revealed about 2400 protein spots. Among those 301 spots with meaningful changes along with the developmental stages, were identified by peptide mass fingerprinting (PMF).

153. **Transcriptome analysis during sexual development in *Aspergillus nidulans*.** Yeong Man Yu[1], Yong Jin Kim[1], Dong-Min Han[2], Kap-Hoon Han[3], Hee-Moon Park[1], Suhn-Kee Chae[4], Kwang-Yeop Jahng[5], Keon-Sang Chae[5], Jong-Hwa Kim[3], Tae Jeong Oh[6], and Pil Jae Maeng[1*]. [1]Chungnam National Univ., Daejeon; [2]Wonkwang Univ., Iksan, Chonbuk; [3]Woosuk Univ., Wanju, Chonbuk; [4]Paichai Univ., Daejeon; [5]Chonbuk National Univ., Chonju, Chonbuk, [6]GenomicTree Inc., Daejeon, Korea

In *Aspergillus nidulans*, although its genome has been fully sequenced, microarray analysis is still in its infancy. Here, we describe analysis of microarrays for *A. nidulans* using a 70-base-oligomer microarray that assays 10,580 representing ORFs from. To estimate the relative gene expression levels and changes in gene expression during sexual development, we analyzed the microarrays throughout a time course during sexual development using a AnURR (*A. nidulans* Universal Reference RNA) in all hybridization. In the oligomers designed for annotated genes, we obtained 9,698 reliable genes (92%). The array revealed time-dependent expression of distinct genes set. The most significantly regulated genes ($P < 0.01$) were grouped in six clusters based on their expression profile. Functional group of the genes contained in each cluster were analyzed by using the MIPS classification. We found several groups of genes that are expected to be involved in the regulation of sexual development at transcriptional level.

154. **Microarray profiling of gene expression of *Aspergillus nidulans* in response to hypoxic stress**. Yeong Man Yu[1], Hye Jun Yun[1], Dong-Min Han[2], Kap-Hoon Han[3], Hee-Moon Park[1], Suhn-Kee Chae[4], Kwang-Yeop Jahng[5], Keon-Sang Chae[5], Jong-Hwa Kim[3], Tae Jeong Oh[6], and Pil Jae Maeng[1*]. [1]Chungnam National Univ., Daejeon; [2]Wonkwang Univ., Iksan, Chonbuk; [3]Woosuk Univ., Wanju, Chonbuk; [4]Paichai Univ., Daejeon; [5]Chonbuk National Univ., Chonju, Chonbuk, [6]GenomicTree Inc., Daejeon, Korea

In all aerobic organisms, oxygen is the terminal electron acceptor in the respiratory chain and is required for ATP generation via oxidation phosphorylation. Thus hypoxia is expected to affect the expression of many genes involved in cellular bioenergetics, sterol, heme, and fatty acid synthesis. In order to figure out the change of transcriptome under hypoxic conditions, we analyzed the profiles of mycelia exposed to hypoxic stress by using a 70-base-oligomer microarray. The array revealed hypoxia-specific expression of distinct genes set. The most significantly regulated genes ($P < 0.01$) were grouped in four clusters based on their expression profile. The clusters analyzed by using the MIPS classification. Our transcriptional profiling data seems to correlate

well with the change in biochemical and physiological processes caused by the hypoxic stress. Several groups of genes expected to be involved in the regulation of hypoxic genes were found.

155. **Gene clusters for secreted proteins in smut fungi: Determinants for the biotrophic lifestyle**. Jan Schirawski, Thomas Brefort, Julia Schöning, Kerstin Schipper and Regine Kahmann. Max Planck Institute for Terrestrial Microbiology, 35043 Marburg, Germany

The two related basidiomycetes *Ustilago maydis* and *Sporisorium reilianum* cause smut disease in maize. Upon penetration of the maize cuticle both fungi proliferate extensively within the plant apoplast. While *U. maydis* induces tumors filled with fungal spores on all aerial parts of its host, spore formation in *S. reilianum* occurs without prior tumor induction exclusively in the inflorescence. To define determinants of compatibility, we focused on the predicted secretome. In *U. maydis* a surprising number of secreted protein-encoding genes occur clustered. These clusters seem to encode virulence factors, since deletion of individual clusters in *U. maydis* in many cases affected pathogenicity. To define conservation/divergence between *S. reilianum* and *U. maydis* potentially explaining differences in disease progression, we sequenced the genome of *S. reilianum* and compared the cluster loci. In *U. maydis* deletion of the largest cluster (19A) containing 23 genes for potentially secreted proteins resulted in loss of tumor induction and spore formation without affecting preceding developmental stages. A detailed microscopic analysis of the cluster 19A deletion mutant in planta suggested recognition by the plant defense machinery at late stages of development. Intriguingly, interspecies comparison of the cluster loci revealed significantly lower conservation between cluster genes than between neighboring genes. These findings suggest that the secreted cluster proteins are under diversifying selection, potentially arising from the different modes of disease development. Currently we determine localization of secreted proteins of *U. maydis* cluster 19A in planta and analyze the role of the corresponding genes during biotrophic development of *S. reilianum*.

156. **Functional characterization of acetylglutamate synthase and phosphoribosylamine-glycine ligase genes in *Gibberella zeae*.** Jung-Eun, Kim[1], Kilseon Myong[1], Won-Bo Shim[2], Sung-Hwan Yun[3], and Yin-Won Lee[1]. [1]School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea [2]Department of Plant Pathology and Microbiology, Texas A&M University, USA [3]Department of Biological Resources and Technology, Soonchunhyang University, Asan 336-745, Korea

Gibberella zeae (anamorph, *Fusarium graminearum*) is an important pathogen of cereal crops in many regions of the world. In this study, we characterized two auxotrophs (designated S4B1279 and S4B3008) that were recovered from a collection of insertional mutants of *G. zeae* created by the restriction enzyme-mediated integration (REMI) procedure. Both mutants showed pleiotropic phenotypes including reduction in mycelial growth and virulence, and no sexual reproduction. Molecular analyses of the REMI mutations revealed that genes responsible for the arginine and adenine auxotrophy in the strains S4B1279 and S4B3008 are *ARG2* (encoding a putative acetylglutamate synthase) and *ADE5* (encoding a phosphoribosylamine-glycine ligase), respectively. Subsequent gene deletion and complementation analyses confirmed the functions of *ARG2* and *ADE5* in *G. zeae*. In addition, complementation of the auxotrophs with intact copy of *ARG2* or *ADE5* indicated that both *ARG2* and *ADE5* can be used as new dominant selectable markers for *G. zeae*

157. **Symbiosis Insights from the Genome of the Mycorrhizal Basidiomycete *Laccaria bicolor***. Francis Martin, Joint Genome Institute and Laccaria Genome Consortium (fmartin@nancy.inra.fr)

Laccaria bicolor is a ubiquitous fungal symbiont of trees in boreal and temperate forest ecosystems. This biotroph has a beneficial impact on plant growth, yet our understanding of the interactions driving this ectomycorrhizal association are poorly known. To elucidate the genetic basis of this ecologically important behavior, we have sequenced the 65 Mb *L. bicolor* genome The genome assembly contains about 20,000 predicted protein-encoding genes and a very large number of transposons and repeated sequences (i.e. 21% of the assembled sequence) which contribute to its large size compared to known fungal genomes. Analysis of this gene set yields insights into unexpected aspects of the symbiont biology including the identification of genes potentially associated with saprobic organic matter decay and pathogenesis. This fungus also possesses expanded protein families associated with plant interactions, such as hydrolases, signaling proteins, and small secreted proteins. WD40 repeat- and TPR-containing proteins – key integrators of stress and nutrient availability signals - are very abundant. Comparison of the genomes of pathogenic and saprobic fungi with the *L. bicolor* genome reveals that the symbiont versatile genome encodes all the features corresponding to the major fungal lifestyles, i.e., saprophytism, pathogenesis and symbiosis. I will close with some thoughts on other strategies, including transcriptomics and proteomics, we are undertaking to fully appreciate the molecular basis of fungal symbiosis.

158. **Browsing *C. albicans* Genomic Information at the *Candida* Genome Database.** Martha B. Arnaud, Maria C. Costanzo, Marek S. Skrzypek, Prachi Shah, Gail Binkley, Stuart R. Miyasato, and Gavin Sherlock. *Candida* Genome Database, Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305- 5120

Candida Genome Database (CGD, freely accessible at http://www.candidagenome.org/) contains genomic sequence data and information about the genes and gene products of the opportunistic human fungal pathogen *Candida albicans*. CGD supports genome viewing and browsing using GBrowse, an open-source tool developed as part of the Generic Model Organism Database project (http://www.gmod.org/). CGD provides access to the two most current versions of the *C. albicans* genome sequence, Assemblies 19 and 20. The Assembly 19 contigs and the Assembly 20 chromosomes are independently navigable. The tool displays a user- configurable set of Tracks, which each show a class of features, for example ORFs, tRNAs, or contigs. CGD has generated sequence-based mappings of the contigs from Assemblies 4, 6, and 19 and the ORFs from Assemblies 6 and 19, onto the chromosomes from Assembly 20, and mappings of the older assembly information onto the Assembly 19 contigs. These historical tracks are available in GBrowse, where they serve as a graphical representation of the evolution of the genome assembly. GBrowse may also be used to view the results of any BLAST query. To view the genomic context of the sequence match, select the link from CGD BLAST results page. GBrowse also allows downloading of any selected region of DNA or protein sequence, annotation of restriction sites recognized by a configurable list of restriction endonucleases, and export of publication-quality images. CGD curators welcome comments and suggestions by email, at candida-curator@genome.stanford.edu. CGD is supported by grant RO1 DE15873 from the NIDCR at the NIH.

159. **Determining mode of antifungal activity of phytochemicals using *S. cerevisiae* and genetic array technologies**. Nadereh Hannah Mir-Rashed[1], Imelda J. Galvan[2], John T. Arnason[3], Myron L. Smith[1]. [1] Biology Department, Carleton University, Canada.[2] Chemistry Department, Universidad Autónoma de Aguascalientes, México. [3] Biology Department, University of Ottawa, Canada.

Host toxicity, a limited spectrum of activity, and propensity to induce or select for resistant fungal pathogens by many current antifungals has created a need for additional agents to treat fungal infections. We have studied how *S. cerevisiae* responds to sub-lethal exposures of plant-derived compounds, including the alkaloid berberine, cinnamodial, chimaphilin and those in Echinacea extracts. DNA microarray analysis of yeast grown in the presence of berberine revealed significant differential RNA transcript levels for 106 genes which were assigned to seven categories including cell cycle/division, metabolism, cell wall, gene expression, transport/secretion and unknown functions. Based on this transcriptional response, we hypothesize that berberine is an effective antifungal because, at least in part, it is not apparently recognized as a xenobiotic. A yeast Gene Deletion Array (GDA, ~4600 gene deletion mutants) was also used to examine chemical-genetic interactions with yeast exposed to each compound. These experiments provide insight into antifungal mode of action and thus add to the value of these bioactive compounds. For example, a significant pattern to emerge from the GDA analysis was that Echinacea extracts interfere with fungal cell wall functions. Furthermore, the experiments provide a template in medicinal plant research. The future direction of the study is to link the collected data to the antifungal drug discovery and development processes.

160. **Telomere instability in *Magnaporthe oryzae* caused by highly mobile, telomere-targeted tranposons**. Mark Farman & John Starnes. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546, USA

Magnaporthe oryzae isolates that infect perennial ryegrass have unusually unstable telomeres that undergo continual rearrangements in culture and *in planta*. Sequencing revealed that the chromosome ends of the ryegrass pathogens are organized very differently to those of a rice-infecting strain with stable telomeres. Specifically, the subtelomeres consist of tandem arrays of two types of repetitive elements, with canonical telomere repeats at the ends of the arrays. The elements are found only in subtelomeric locations, so we have named them *M. oryzae* telomere- exclusive repeats (MoTERs). MoTER1 is 4.6 kb in length and codes for a reverse transcriptase. It lacks terminal repeats and, therefore, appears to be a non-LTR retrotransposon. MoTER2 is only 1.7 kb long and potentially codes for a 204aa protein of unknown function. We have identified two new insertions of MoTER1 at telomeres that originally lacked MoTER elements, thereby demonstrating that MoTER1 is an active transposon. The structures of MoTER1 and MoTER2, and the way they are organized at the chromosome ends, are reminiscent of the retrotransposons TART and HeT-A, which are responsible for maintaining the Drosophila telomeres. Therefore, we hypothesize that *M. oryzae* strains from ryegrass have dual systems for telomere maintenance – one based on telomerase, and another which uses terminally targeted retrotransposons to repair degraded ends that arise during telomere crisis. Ironically, it appears that the presence of the MoTER elements causes the telomeres to be unstable in the first place – an apparent case of a selfish element working hard to ensure the security of its own job!

161. **mRNA profiling revealed enrichment of orphan gene transcripts during asexual development of a filamentous ascomycete *Neurospora crassa***. T. Kasuga[1], H. H. Wilkinson[2], D. Ebbole[2], B. Shaw[2], G. Mannhaupt[3], J. Taylor[1] and L. Glass[1]. [1]Dept. Plant & Microbial Biology, Univ. California, Berkeley, CA. [2]Dept. Plant Pathology & Microbiology, Texas A&M Univ., College Station, TX. [3]MIPS, Neuherberg, Germany.

We asked if there is any correlation between the pattern of expression and phylogenetic age for *Neurospora crassa* genes. *N. crassa* genes were classified into six mutually exclusive groups (Cai et al., J. Mol. Evol 2006): (1) Eukaryote/Prokaryote-core, a group of genes whose orthologs were identified in non-fungal Eukaryotes and/or Prokaryotes, (2) Basidiomycota-core, genes shared with basidiomycetes, (3) Hemiascomycete-core, (4) Euascomycete- specific, (5) *N. crassa* orphans, which lack sequence

similarity to any of the genes in SIMPS database and (6) Remainder. We then examined the mRNA profiles obtained during vegetative growth and conidiation using *N. crassa* microarray representing all the predicted ORFs (c.a. 11,000). We found an over-representation of Euascomycete-specific genes in very young hyphae (0 to 3 hours old) and in mature hyphae engaged in conidiation (18 to 27 hours old; enrichment test, $p < 0.01$). On the other hand, Eukaryote/Prokaryote-core genes showed peak expression pattern in 6 to 15 h old hyphae. The most notable finding was the expression timing of *N. crassa* orphan genes. There are c.a. 2,000 orphan genes in the *N. crassa* genome and 95% of them do not have functional annotation. *N. crassa* orphan genes were over-represented during development of aerial hyphae and conidiation, which coincided with development of the conspicuous and distinctive asexual morphology characteristic of this fungus.

162. **Functional analysis of genes regulated by *MAT1-2* in *Gibberella zeae* using a proteomics approach.** Seung-Ho Lee[1], Sung-Hwan Yun[2], and Yin-Won Lee[1]. [1]School of Agricultural Biotechnology, Seoul National University and Center for Agricultural Bio-materials, Seoul 151-921; [2]Department of Biological Resources and Technology, Soonchunhyang University, Asan 336-745, Korea

The homothallic ascomycetes *Gibberella zeae* (anamorph = *Fusarium graminearum*) is an important pathogen with ubiquitous geographic distribution and causes severe diseases on cereals. Ascospores formed within the sexual fruiting body (perithecium) of *G. zeae* can overwinter and initiate the primary infection in the next spring. Thus, a greater understanding of sexual development in *G. zeae* is needed for a comprehensive control strategy of the diseases caused by this fungus. We have focused on identifying the protein specifically controlled by *MAT1-2*, a master regulator of sexual reproduction in *G. zeae*. To select differentially expressed proteins under control of *MAT1-2* during the perithecial stage, we compared the protein profiles of a self-fertile *G. zeae* and its isogenic strain deleted for *MAT1-2* using a two-dimensional electrophoresis. Total 384 protein spots were visualized by silver staining and 12 spots were differentially expressed during sexual reproduction mode; only two proteins were highly expressed in the *MAT1-2* deletion mutant. We identified genes corresponding to these proteins from the genome database and determined their gene expression patterns and functional requirement for sexual development. Of 13 genes examined, 10 genes were essential for either perithecia or ascospore formation in *G. zeae*. This study is the first report on functional analyses of a set of genes controlled by *MAT1-2* at the protein level.

163. **Genetic questions that we expect to answer by sequencing the genome of the white-rot basidiomycete *Pleurotus ostreatus.*** Antonio G. Pisabarro, Lucía Ramírez Public University of Navarra, Pamplona, Spain. E-mail: gpisabarro@unavarra.es

The sequencing of the *P. ostreatus* genome been scheduled for 2007 by the JGI. *P. ostreatus* has been studied as edible fungus, as model for lignin degradation and as source of poly-aromatic hydrocarbon degrading enzymes. However, the genetic questions that can be addressed using this organism as model are far beyond that. This organism is dikaryotic in all its life cycle except at the basidia. This allows separating the two nuclei present in a mature sexually competent individual to study both parental haplotypes. Electrophoretic analyses of *P. ostreatus* have revealed prominent length polymorphisms in homologous chromosomes. The sequence of the two sets of homologous chromosomes will make available the two haplotypes of a mature organism for the first time. This will permit to determine the minimal synteny between homologous chromosomes and to study the meiotic behaviour of structurally polymorphic chromosomes. In *P. ostreatus* various quantitative loci (QTLs) have been identified whose genetic architecture is poorly known. The sequence of the two haplotypes will permit to study this architecture and to evaluate the contribution of other genomic regions interacting with it. Finally, the study of the contributions of the two haplotypes to subcellular structures (nucleus, mitochondria) will be possible, and this will open the door to deeper analyses

164. **Phenotypic and metabolomic analyses of the *Fusarium graminearum snf1* mutant.** Martin Urban, John Antoniw, William Allwood, Andrew Beacham, Jane Ward, Mike Beale and Kim Hammond- Kosack. Rothamsted Research, Herts, AL5 2JQ, UK; Kim.Hammond-Kosack@bbsrc.ac.uk

The Ascomycete fungus *Fusarium graminearum* (Fg) is a ubiquitous plant pathogenic fungus in all major cereal growing areas of the world. Infections, which occur during plant anthesis, lower grain quality and contaminate grains with trichothecene mycotoxins. The genome was sequenced by the BROAD Institute and completely aligned to the genetic map. Fg has 4 chromosomes and minimal repetitive DNA (http://www.broad.mit.edu/annotation/fungi/fusarium) . In an effort to identify the set of pathogenicity genes in Fg and other fungi, a database of known fungal and Oomycete pathogenicity genes was established at Rothamsted Research (http://www.phi- base.org) . We have identified all the Homologues of verified pathogenicity and virulence (HvPV) genes in Fg, and created a chromosome visualisation software, called Mycomap to inspect visually the chromosomes for clustering of HvPV genes. This analysis identified a region on chromosome 1, where 5 HvPV genes are clustered together within 24 kb. This includes the *S. cerevisiae SNF1* gene homologue, a regulator of catabolite derepression in yeast and a regulator of cell wall degrading enzymes in *Cochliobolus carbonum* and *F. oxysporum.* We deleted the *snf1* gene in Fg as the first member of the HvPV gene cluster in chromosome 1 to characterise the importance of catabolite derepression during plant pathogenicity and mycotoxin production. The availability of the *snf1* mutant, which exhibits highly reduced virulence on wheat ears, has allowed us to use 1H-NMR metabolomic analysis as a tool to identify fungal candidate metabolites

with a role in plant pathogenicity. Rothamsted Research receives grant aided support from the Biotechnology and Biological Sciences Research Council.

165. Withdrawn

166. **A whole genome oligo microarray approach to decipher *Podospora anserina* sexual development.** Frederique Bidard [1], Sandrine Imbeaud [2], Sylvie Arnaise[1], Anne Bourdais[1], Karine Budin[2], Evelyne Coppin[1], Eric Espagne[1], Olivier Lespinet[1], Fabienne Malagnac[1], Mathieu Paoletti[3], Leonardo Peraza- Reyes[1], Nancy Reymond[2], Sven Saupe[3], Eliette Sicault-Sabourin[3], Denise Zickler [1], Philippe Silar[1], Corinne Clave[3], Herve Delacroix[2], Veronique Berteaux-Lecellier[1] and Robert Debuchy[1]. [1]Institut de Genetique et Microbiologie, Univ Paris-Sud, UMR8621, F-91405 Orsay, France. [2]Centre de Genetique Moleculaire, GODMAP, CNRS UPR 2167, F-91198 Gif sur Yvette, France. [3]Institut de Biochimie et Genetique cellulaire, Univ Bordeaux2, UMR 5095, F-33077 Bordeaux, France.

The sexual development of the heterothallic euascomycete *Podospora anserina* has been analyzed for many years in terms of signal transduction pathways controlling the switch from vegetative to reproductive state, autophagy requirement for fruiting-body maturation, functions of MAT proteins and the developmental role of peroxisomal proteins. The completion of the *P. anserina* genome sequence allows all these research areas to benefit now from a microarray approach. Agilent 22K microarrays targeted against the 10,700 putative CDS of *P. anserina* will be used to compare wild type and the numerous mutants obtained in this model–system. This project is funded by Agence Nationale de la Recherche, grant n° ANR-05-0385-01.

167. **Transcriptional Analysis of the Pathogenic Fungus *Magnaporthe grisea* during rice infection** Cécile Ribot*, Arnaud Lagorce***, Aurélie Darchis, Fabien Munier, Jean-Benoit Morel**, Rick De Rose*, Roland Beffa* and Marc-Henri Lebrun*. *Bayer CropScience / CNRS, 69263 Lyon Cedex 09, France **CIRAD, TA73/09, 34398 Montpellier Cedex 05, France ***Institut de Genetique et de Microbiologie, Laboratoire de Genomique des Archaea, Université Paris XI / UMR8621 CNRS, 91405 ORSAY Cedex, 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 tools (genome sequence, EST databases, DNA chip, forward and reverse genetics) are available. Using Agilent 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 rice cultivar IR64 were infected by the virulent *M. grisea* strain PH14. Infected rice leaves were harvested 5 days after infection at the onset of lesions. 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 1800 genes that are expressed during infection. Among these 1800 genes, 240 genes are specifically expressed during infection, as no signal was detected in a mycelium array. 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 for 75% of the genes. Interestingly, bioinformatic analyses revealed an over representation of genes encoding for proteins putatively secreted. While only 5 % of the proteins encoded by the whole genome of *M. grisea* are predicted to be secreted, 43 % (105/240) of the protein specifically expressed during the infection process a signal peptide for protein secretion. These results suggest that *M. grisea* may produce numerous secreted effector proteins in host tissues that could be involved in the infection process. A systematic analysis of *M. grisea* proteins secreted during infection has been started to study this process.

168. **Relative Protein Quantification through Stable Isotope Labeling by Amino Acids in *Aspergillus flavus*: Temperature Regulation of Aflatoxin Biosynthesis.** D. Ryan Georgianna[1,3], David C. Muddiman[2], and Gary A. Payne[1]. [1]Department of Plant Pathology and Center for Integrated Fungal Research. [2]Department of Chemistry. [3]Functional Genomics Graduate Program. North Carolina State University,Raleigh, NC 27606 USA.

Aflatoxin biosynthesis is inhibited at 37C, the optimum temperature for growth of *Aspergillus flavus*. Transcriptional analysis has shown that all aflatoxin biosynthetic genes except the pathway regulatory genes *aflR* and *aflS* are down regulated at 37C relative to 28C, suggesting that AFLR may be modified at 37C. To quantify the response of AFLR and other proteins to high temperature we adapted a stable isotope labeling by amino acids (SILAC) strategy for relative protein quantification in *A. flavus*. SILAC relies on the quantitative incorporation of labeled amino acids into proteins to provide a powerful mass spectrometry based proteomics tool useful for both the rapid quantification of proteins and identification of interactions. This technique has been used in several systems; including yeast, mammalian cells, and *Arabidopsis* cell culture. Samples were prepared using in-gel trypsin digestion of selected 1D-PAGE gel slices and analyzed on an ESI LTQ linear ion trap mass-spectrometer directly coupled to RP- HPLC. The *A. flavus* labeling strategy was optimized to provide a homogenously labeled sample with ~90% incorporation of [13C6] arginine. Furthermore, we found that the relative abundance of aflatoxin pathway enzymes compared between 28C and 37C is consistent with the relative abundance of their encoding transcripts at the respective temperatures. This is the first report of SILAC being used to quantify proteins in a filamentous fungus as well as a multi- cellular free-living prototrophic organism.

169. **Comparative analysis of the cell wall sub-proteome from genetically-related strains of *Cryptococcus neoformans* with distinct pathogenic properties.** Jamal Stie and Deborah Fox. Research Institute for Children/LSUHSC. Pediatrics and Microbiology, New Orleans, LA

Fungal infections are a frequent and growing cause of morbidity and mortality in the immuno-suppressed patient population, with effective treatment and continued antifungal drug development compromised by the conservation, at the molecular level, of many key physiological processes common to both humans and fungi. However, fungi elaborate one unique structure that is essential for their survival, the fungal cell wall, which acts as a physical barrier, and mediates events necessary for environment sensing, growth and morphogenesis. While the carbohydrate and protein composition of the cell wall has been extensively studied for many fungi, the cell wall of *Cryptococcus neoformans*, a significant opportunistic fungal pathogen that causes life-threatening meningoencephalitis is largely uncharacterized. Recent efforts to sequence and annotate the genome of this pathogen revealed the 99.5% genetic identity of two related strains of *C. neoformans* that possess markedly different virulence phenotypes, with the B3501A strain considerably more virulent than JEC21. Because the protein population within the cell wall plays an important role in the mediation of virulence in other pathogenic organisms, we hypothesize that differences in the cell wall protein composition could contribute to differences in virulence. Here we examined the hypothesis that B3501A and JEC21 differentially express proteins within the cell wall sub-proteome, contributing to the differences observed in the virulence properties of the two strains. Using a directed proteomics approach, we have performed a comparative analysis of the cell wall sub-proteomes from each strain, revealing a small subset of proteins that are unique to each, suggesting the presence of strain-specific cell wall-associated virulence determinants. Ongoing studies utilizing LC-MS/MS will permit the identification of both common and unique protein populations within the cell wall sub-proteome for each strain, leading to the elucidation of important surface-associated mediators of host interaction during pathogenesis in *C. neoformans*.

170. **PhESTDB: An Integrated resource for *Phytophthora* and Soybean EST sequences**. Sucheta Tripathy and Brett M. Tyler. Virginia Bioinformatics Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA-24061 sutripa@vbi.vt.edu bmtyler@vt.edu

PhESTDB is an unique integrated EST data resource, containing 33,350 *P.sojae* ESTs, 99,319 *P.infestans* ESTs and 37,465 public Soybean unigenes. This database is the most complete resource for *P.sojae* EST sequences to date. The *P.sojae* sequences were quality trimmed followed by vector/ adaptor, poly A/T trimming. A total of 3769 Soybean EST sequences were separated from the *P.sojae* ESTs from an infection library using in silico methods. Subsequently, the sequences were clustered and assembled using a wrapper perl script TGICL.The clustering and assembly produced 7863 unigenes of *P.sojae*, 2292 soybean unigenes from the infection library, and 14754 unigenes of *P.infestans*. The unigenes were primarily annotated using NCBI-BLASTX against NR database accelerated with a Timelogic board.The unigenes were then passed through a modified algorithm of loglikelihood(McLachlan,1984) to separate the UTRs from the ORFs. The protein sequences were annotated for domains, motifs, profiles and fingerprints using InterproScan. Sequences were submitted to signalp and TMHMM servers to get secretory and trans-membrane domains. The resulting outputs were parsed and stored in the database. The database was designed in mysql and the interfacing was done with PHP and perl CGI. We have created an easy-to-use Perl GD-based browser which is distinct from Gbrowse. In the browser the genome to unigene alignments are shown in greater detail which also indicates any overlaps between the unigenes. Each EST unigene is made clickable to view detailed information about assembly and their primary annotation including various other related information such as NCBI links and links to gene models. Through the gene models this database is linked to the VBI microbial database. This database has a very powerful query page, that can retrieve any kind of information the user may need. Data can also be downloaded from the database in text format. The database is publicly available at http://phytophthora.vbi.vt.edu/EST .

171. **Sequencing of the genome of the forest pathogen *Heterobasidion annosum* s.l.** Jan Stenlid[1], Matteo Garbelotto[2], Ursula Kües[3], James B Anderson[4], Francis Martin [5], Halvor Soilheim[6]. [1]Swedish University of Agrcultural Sciences, Uppsala, Sweden, [2] University of California, Berkeley, USA, [3] Georg August University of Göttingen, Germany, [4]University of Toronto, Canada, [5] INRA-Nancy, France [6] Norwegian University of Life Sciences, Ås Norway

Heterobasidion annosum s.l. causes a devastating root rot in conifer plantations and natural forests throughout the northern hemisphere. The JGI- sponsored genome sequencing for *H. annosum* will provide the first comprehensive genetic information on a plant pathogenic homobasidiomycete allowing for new insights into plant- microbe interactions with trees, in particular conifers. It is important to broaden the taxonomic base for understanding the mechanisms of plant-microbe interactions, studying genes and proteins involved, and identifying pathogenicity determinants. Comparative genomics of plant pathogens with a gradient of taxonomic relatedness to *H. annosum* will help to understand the evolution of such factors. Comparisons can also be made in the response of plants to various types of trophic interactions; necrotrophic, biotrophic and mycorrhizal mutualists and the model tree *Populus*. Moreover, this project will also gain insights into fungal evolutionary history and biology including development, non self recognition, mating, wood degradationand secondary metabolism.

172. **High throughput discovery of virulence factors among transcription factors of *Alternaria brassicicola.*** Yangrae Cho, Mauricio La Rota, Kwang-Hyung Kim, Derrick Scott, Amanda Cronin, Meagan Callilhan, Graciela Santopietro, and Christopher Lawrence. Virginia Tech, Blacksburg, VA

Alternaria brassicicola is a ubiquitous necrotrophic fungus causing destructive rots among diverse Brassica family members. We have optimized relatively easy and economical PEG-mediated protoplast transformation methods for this fungus. In order to produce targeted gene knockout (KO) mutants, we have utilized unconventional linear minimal element constructs as well as conventional gene replacement constructs. Using these two methods, we produced knockout mutants for over 100 genes and investigated changes in virulence. Here we present the results related to mutants for 40 protein coding genes. These candidates were comprised of five kinases and four transcription factors (TFs) involved in six known signal transduction pathways. In addition 31 TFs from the partially annotated *A. brassicicola* genome were also selected (both randomly and based on interesting predicted functional domains) for KO and subsequent phenotypic analyses. Near loss of virulence was observed for mutants of the *Amk1*, *AbSte12*, and *AbMps1* genes, representing two signal transduction pathways of the mating pheromone responses and hypotonic shock responses. Significant reduction of virulence without any other discernable phenotypes has been observed for mutants of the *AbPro1* gene whose homolog is involved in sporulation in diverse filamentous fungi. In addition, we have discovered mutants for two of the 31 selected TF genes showed reduced virulence. The study results thus far show a few common as well as several unique aspects of virulence mechanisms in *A. brassicicola*.

173. **The genome of the necrotrophic fungus *Alternaria brassicicola*.** C. Mauricio La Rota[1], Yangrae Cho[1], Thomas Mitchell[2], Brett Tyler[1], Sandra Clifton[3], Dennis Knudson[4], Susan Brown[4], Christian Haudenschild[5], Blake Meyers[6] and Christopher Lawrence[1]. [1] Virginia Bioinformatics Institute, Blacksburg, VA. [2] Fungal Genomics Lab, North Carolina State University, Raleigh, NC. [3] Washington University Genome Sequencing Center, St Louis, MO. [4] Colorado State University, Fort Collins, CO. [5] Solexa Inc, Hayward, CA. [6] Delaware Biotechnology Institute, Newark, DE.

The genomes of several fungi within the Dothideomycete are in the process of being sequenced at various centers internationally. These fungi are economically important plant pathogens infecting various important crops such as wheat, corn, and Brassicas, to name a few. All of these fungi are capable of leading a saprophytic lifestyle but have a necrotrophic stage in their lifecycle. Comparisons between their gene contents and genome structures are likely to reveal important details about their evolutionary history, production of toxins and the capability to switch from a saprophytic phase to a necrotrophic one in order to infect plant tissues. We present here the first draft assembly of the 30Mb genome of the Brassica pathogen, *Alternaria brassicicola* with a 6.4x coverage and provide details about the progress in annotating the genome, the manual curation and the functional genomics aspect of the project. Analysis of the genome features and structure will help to further the understanding of necrotrophic pathogen-plant interactions. This project is funded by USDA-CSREES (Proj. # VAR-2004-05551).

174. **Comparative and functional genomics in identifying aflatoxin biosynthetic genes.** Jiujiang Yu[1,7], Jeffery Wilkinson[2], Gary Payne[3], Masayuki Machida[4], Bruce Campbell[5], Joan Bennett[6], Deepak Bhatnagar[1], Thomas Cleveland[1], and William Nierman[7,8]. 1USDA/ARS, Southern Regional Research Center, New Orleans, LA 70124, USA; 2Mississippi State University, Mississippi State, MS 39762, USA; 3North Carolina State University, Raleigh, NC 27695, USA; 4National Institute of Advanced Industrial Science and Technologies (AIST), Tsukuba, Ibaraki, Japan; 5USDA/ARS, Western Regional Research Center, Albany, CA 94710, USA; 6Rutgers University, New Brunswick, NJ 08901, USA; 7The Institute for Genomic Research, Rockville, MD 20850, USA; 8The George Washington University School of Medicine, Washington, DC 20037, USA

Identification of genes involved in aflatoxin biosynthesis through *Aspergillus flavus* genomics has been actively pursued. *A. flavus* Expressed Sequence Tags (EST) and whole genome sequencing have been completed. Groups of genes that are potentially involved in aflatoxin production have been profiled using microarrays under different culture conditions and during fungal infection of corn. Preliminary annotation of the sequence revealed that there are about 12,000 genes in the *A. flavus* genome. Many genes in the genome, which potentially encode for enzymes involved in secondary metabolite production, such as polyketide synthases, non-ribosomal peptide synthases, cytochrome P450 monooxigenases, have been identified. Comparative analysis of *A. flavus* genome with food grade industrial fermentation organism *A. oryzae* can help understanding the mechanism of aflatoxin biosynthesis and solving the problem of aflatoxin contamination.

175. **Validation of DNA microarrays derived from the *Fusarium verticillioides* Gene Index.** Robert A.E. Butchko, Daren W. Brown and Robert H. Proctor. Mycotoxin Research Unit, National Center for Agricultural Utilization Research, 1815 N. University Street, Peoria, IL 61606

Fusarium verticillioides is a pathogen of maize and it can produce the toxic polyketide derived secondary metabolites called fumonisins. Fumonisins have been shown to cause animal diseases and are epidemiologically correlated to esophageal cancer and neural tube defects in humans. In an effort to identify genes involved in regulation of fumonisin production and in pathogenicity on maize, a large scale EST sequencing project was conducted in collaboration with The Institute for Genomic Research. The *Fusarium verticillioides* Gene Index (FvGI) contains up to 11,126 sequences including 7198 tentative consensus (TC) and 3921

singleton EST sequences. DNA microarrays were designed based on the FvGI and were constructed by NimbleGen Systems, Madison WI. Each DNA microarray contains 12 different 24-bp oligonucleotide probes specific to each of the unique sequences in the FvGI. Here, we present data from an experiment where total RNA from a six-point time course of *F. verticillioides* cultured in a liquid medium conducive to fumonisin production was used to hybridize to the microarrays. Analysis of *FUM* gene expression patterns over time were used to validate the microarray. The expression of *FUM* genes determined by the microarray analysis is consistent with expression patterns determined by Northern analysis and RT-PCR. To help determine the orientation of singleton ESTs whose direction could not be determined from sequence data or BLAST analysis, probe sets to both plus and minus strand sequences were included for all of the singletons as well as TCs containing two open reading frames. Microarray analysis successfully resolved the orientation of a number of the singletons for which the orientation was not obvious and demonstrated the existence of two open reading frames in some TCs. Global patterns of gene expression under these conditions are also presented.

176. **Measuring LOH rates in *Candida albicans* by fluctuation analysis.** Anja Forche [1], Merima Helić [1], and Judith Berman[1]. [1]University of Minnesota, Minneapolis, MN, USA e-mail: forche@umn.edu

Candida albicans is the most common human fungal pathogen. While it does not undergo meiosis, clinical strains exhibit high levels of genetic and genomic variability that can lead to the development of drug resistance. To explore the mechanisms that give rise to genetic and genomic changes, we have measured basal rates of recombination by following loss of heterozygosity (LOH) in fluctuation assays. We used two counter-selectable genetic markers, *GAL1* and *URA3*, located on opposite alleles at the same locus on chromosome 1. Basal LOH rates were approximately 10^{-6} events/cell/generation for each marker, indicating that the assay is effective, that it is independent of the marker used and that both homologues of chromosome 1 undergo equal rates of LOH. SNP microarrays were used to study the extent and the mechanisms of LOH along chromosome 1 in strains that had undergone LOH at *GAL1*. Eighty-six percent of LOH events were caused by a single cross-over event that extended to the telomere, most likely due to break-induced replication. Shorter gene conversion tracts (9%) and LOH of whole chromosomes (5%) also were observed. We are currently testing if 1) the basal LOH rate is altered in the presence of anti-fungal drugs and other stresses such as temperature and toxic agents, and if 2) LOH rates differ at different chromosomal loci including telomeres, centromeres and mid-arm loci on all eight chromosomes. Preliminary results suggest that LOH is much higher at telomeres than at centromeres or internal chromosomal loci.

177. **Creating a Critical Mass of Data for Genome Annotation and Comparative Analysis.** Igor Grigoriev[1*], Andrea Aerts[1], Alan Kuo[1], Asaf Salamov[1], Diego Martinez[2], Jean Challacombe[2], Thomas Brettin[2], Erika Lindquist[1], Harris Shapiro[1], Gregory Werner[1], Susan Lucas[1], Jane Grimwood[3], Jeremy Schmutz[3], Paul Richardson[1], James Bristow[1]. [1]US DOE Joint Genome Institute, Walnut Creek, CA 94598 [2] US DOE Joint Genome Institute - LANL , Los Alamos, NM 87545 [3] US DOE Joint Genome Institute - Stanford, Palo Alto, CA 94304 [*]ivgrigoriev@lbl.gov

The DOE Joint Genome Institute contributes to the rapidly growing number of fungal genomes available to the research community through high-throughput sequencing and annotation. We sequenced and annotated 9 fungal and 2 oomycete genomes (genome.jgi.doe.gov) and expect to nearly double this number in 2007. The broad and diverse set of genomes covers major branches of the fungal phylogenetic tree and samples some of the branches at higher depth, which allows analysis of differences between clades and within species on the genomic level. In addition to several basidiomycete and ascomycete genomes, we have annotated the genome of the zygomycete *Phycomyces blakesleeanus* and are currently annotating the chytridiomycete *Batrachochytrium dendrobatidis*. Many of these genomes are either finished or are in finishing, which enables effective analysis of genome organization and evolution, such as, for example, a conditionally dispensable chromosome in *Nectria haematococca* MPVI. Genome projects triggered development of additional resources of ESTs, proteomics and microarray data to assist with genome annotation and expression analysis. User Communities with over 100 biologists around the world actively participate in analysis and manual curation of predicted genes and functions using the JGI Genome Portal. Creating a critical mass of data improves genome annotation and enables efficient comparative analysis to address a broad spectrum of biological questions and to find important industrial applications.

178. A journey into the unknown: investigating temperature sensitive mutants in *Neurospora crassa*. Kevin McCluskey[1], Sheera A. Walker[1], Rachel L. Yedlin[1], David Madole[2] and Michael Plamann[1,2]. [1]Fungal Genetics Stock Center, and [2]University of Missouri- Kansas City, School of Biological Sciences.

In our continuing effort to bring added value to the materials in the FGSC collection, we have endeavored to identify the open reading frame associated with genetically mapped temperature sensitive lesions in *Neurospora crassa.* There are nearly 60 such ts lesions mapped in the *N. crassa* genome and because there is no function for most of these, they are known as 'unknown'. We used functional complementation of the temperature sensitivity to identify open reading frames for two such lesions, *un-16* and *un-4*. When we identified *un-16*, we found that it had been previously identified by Sakai *et al* (2002) as part of their investigation of the near-by *upr-1*. *un-16* encodes a ribosomal protein and we sequenced the two mutant alleles and found that in both there was a single leucine to arginine substitution. A search of the genome databases revealed that the leucines in question

are very highly conserved- from archae to humans. The ability to complement the temperature sensitive lesion in strains carrying the *un-16* lesion makes it possible to use this as a selectable marker. The second unknown to be complemented, *un-4*, encodes a mitochondrial import protein. Our next target is *un-7* and currently we have identified 8 candidate ORFs from existing data. The FGSC is supported by grant 0235887 from the US National Science Foundation.

179. **Optimised methods for proteome analysis of the encapsulated fungus *Cryptococcus gattii.*** Jocelyne M D'Souza-Basseal[1],Ben R Herbert[2], Matthew Padula[2], Cameron Hill[2], Mark B Krockenberger[3], Elizabeth J Harry[4] and Dee Carter[1]. [1]School of Molecular and Microbial Biosciences, University of Sydney, Sydney, NSW Australia; [2]Proteomics Technology Centre of Expertise, University of Technology, Sydney, NSW Australia; [3]Faculty of Veterinary Science, University of Sydney, Sydney, NSW Australia; [4]Institute for the Biotechnology of Infectious Diseases, University of Technology, Sydney, NSW Australia Contact email: j.dsouza@mmb.usyd.edu.au

The fungal species *Cryptococcus gattii* is an environmental saprophyte capable of causing serious disease in people and animals, and is uniformly fatal without treatment. Like most systemic mycoses, cryptococcosis is difficult to treat and this is due to the scarce number of safe and efficacious antifungal drugs, and to our limited knowledge of the intricacies of fungal virulence. Proteome analysis enables the identification and characterization of the protein complement of a cell, tissue or organism at any one time, under specific conditions. The global nature of proteomics means areas of little knowledge, like fungal disease, can be addressed without any prior knowledge or assumptions about the role of differentially expressed proteins. Furthermore, as proteins are targets for drug interventions in microbial infections, proteome approaches are the most rapid, direct and powerful means of identifying novel candidate antimicrobial targets. The major virulence factor of *C. gattii* is its polysaccharide capsule, composed mainly of glucuronoxylomannan residues and mannoproteins. In our preliminary proteome analysis, an encapsulated *C. gattii* strain was extracted using the current best practice for two-dimensional electrophoresis of microorganisms. However, this gave very limited extraction of high molecular weight proteins and suggested that the capsule may be trapping the 'missing' proteins by ionic interactions. We have developed a novel method of protein extraction using high salt combined with acidic conditions. We present here studies using a range of different salts that greatly increased protein extraction from various encapsulated *C. gattii* strains.

180. **Transcritome analyses of developmental processes associated with hypoxic stress in *Aspergillus nidulans* and *A. fumigatus*.** Kap-Hoon Han[1], Hyoun-Young Kim[1], Jong Hwa Kim[1], Yeong-Man Yu[2], Pil-Jae Maeng[2], Hee-Moon Park[2], Suhn-Kee Chae[3], Keon-Sang Chae[4], Kwang-Yeop Jahng[4], and Dong-Min Han[5]. [1]Dept. of Pharmaceutical Engineering, Woosuk University, Wanju, 565-701, [2]School of Bioscience & Biotechnology, Chungnam Nat'l University, Daejeon, 305-764, [3]Dept. of Life Science & Technology, PaiChai University, Daejeon, 302-735, [4]Div. of Biological Sciences, Chonbuk Nat'l University, Jeonju, 561-756, [5]Dept. of Life Science, Wonkwang University, Iksan, 570-749, Korea.

Aspergillus fumigatus is the primary causative agent of an opportunistic fungal disease, aspergillosis. Unlike *Aspergillus nidulans*, *A. fumigatus* has no known sexual development process. Furthermore, in *A. nidulans*, hypoxic condition is the one of most important environmental factor for generating fruiting bodies. This condition is also important to *A. fumigatus* because of the environment of host cell is usually maintained as hypoxic condition. To study relationship between hypoxic stress, developmental process and virulence, comparative DNA microarray analyses were performed using *A. fumigatus* and *A. nidulans* chips. Comparisons of the two transcriptome provided important information of hypoxic stress response and sexual development process. This work was supported by the KOSEF grant (No. R1-2006-000-11204-0) and KRF (KRF-2005-070-C00123).

181. **Identification of proteins involved in early phase of conidia germination by quantitative proteomics in *Aspergillus nidulans.*** Young Taek Oh*, Chun Seob Ahn, Jae Won Kim, Chang-Won Lee**. Department of Microbiology, Research Institute of Life Science, Gyeongsang National University, 900 Gazwa-dong, Jinju 660-701, Korea *presenting author, **corresponding author

The asexual spore, or conidium of *A.nidulans* become competent for growth and nuclear division in a process called conidial germination, and that begins when the conidium breaks dormancy and growth isotropically. To find out critical proteins involved in early phase of conidia germination, we performed a global proteome analysis of differentially expressed protein between dormant conidia with germinating conidia. More than 1200 protein spots were detected on silver stained 2-D gel with IPG strip pH 4-7. Image analysis revealed that 97 protein spots showed quantitative variations that were significant ($P < 0.05$) and reproducible in the 30minute germinating conidia. And 144 protein spots showed quantitative variations that were significant ($P < 0.05$) and reproducible in the 60minute germinating conidia. Protein identification was carried out peptide mass fingerprinting by MALDI-TOF mass spectrometry. Under 30minute germinating conidia, the protein expression levels of 9 proteins including thioredoxin reductase are up-regulated, and 5 proteins including transketolase are down-regulated. Under 60minute germinating conidia, the protein expression levels of 13 proteins including peptidyl-prolyl cis-trans isomerase are up-regulated, and 12 proteins including mannitol-1- phosphate dehydrogenase are down-regulated. The identified proteins in this report enhance to understanding of the process of conidia germination.

182. **Coding tandem repeats generate genetic diversity in *Aspergillus fumigatus* genes.** Emma Levdansky[1], Jacob Romano[1], Kevin J. Verstrepen[2], Gerald R. Fink[2] and Nir Osherov[1]. Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge, MA[2], Department of Human Microbiology,Sackler School of Medicine[1], Tel-Aviv University, Tel-Aviv, Israel.E-mail: nosherov@post.tau.ac.il

Genes containing multiple coding tandem repeats are highly dynamic components of genomes. In humans, expansion of coding repeats is associated with various diseases, including Huntington's Disease and Fragile X Syndrome. In fungi, many repeat-containing genes encode for cell-wall proteins containing a leader sequence and glycophosphatidylinositol (GPI)- anchor. Several encode for known adhesins (ie. *Saccharomyces cerevisiae FLO11, Candida albicans ALS1, ALS5, ALS7* and *ALS9, Candida glabrata EPA1*). They undergo frequent recombination-dependent expansion or contraction in size, creating size-variability between different yeast isolates and alterations in adhesion. This variation provides the functional diversity in cell-surface antigens which allows rapid adaptation to the environment. *Aspergillus fumigatus* is the most common mold pathogen in man, causing both deadly invasive diseases in immunocompromised patients and allergic diseases in patients with atopic immune systems. We analyzed the entire genome of *A. fumigatus* for open-reading frames (ORFs) containing putative repeats, using the ETANDEM software program, a numerical score for tandem repeats in a nucleotide sequence. Fourteen of 30 top-ETANDEM-scoring genes analyzed showed strain-specific size variation of repeat containing-regions. Four of these, (*Afu3g08990, Afu2g05150 (MP-2), Afu4g09600* and *Afu6g14090*) are putative cell-wall proteins containing a leader sequence and GPI-anchor motif. All four are expressed genes and produce size-variable mRNA encoding a discrete number of repeat amino-acid units. Their expression during development and in response to cell-wall disrupting agents was analyzed. Our findings suggest that a subset of the *A. fumigatus* cell-surface proteins may be hyper-variable due to recombination events in their internal tandem repeats. We have created knock out strains in *A.fumigatus* 293 for two putative cell-wall coding genes (*Afu3g08990* and *Afu6g14090*). We are currently studying the effect of the deletion of these genes on the phenotype. Preliminary results indicate that compared to the wild type strain, *Afu3g08990*-deleted strain exhibits early germination rates and reduced adhesion to ECM (extra cellular matrix) of alveolar lung cells (A549 cells). In addition, we are analyzing *Afu3g08990* and *Afu6g14090* cellular location by tagging the genes with a myc epitope.

183. **Molecular analysis of the response of cultured A549 lung cells to *A. fumigatus* infection.** Haim Sharon and Nir Osherov[1]. Department of Human Microbiology, Sackler School of Medicine,[1] Tel-Aviv University, Tel-Aviv, Israel.E-mail: nosherov@post.tau.ac.il

Aspergillus fumigatus is the most prevalent airborne fungal pathogen which causes fatal invasive aspergillosis in immunocompromised patients. Invasive pulmonary aspergillosis (IPA) is caused by inhalation of *A. fumigatus* spores and growth of the fungus inside the lungs, often spreading from the initial site of infection in the lungs to attack various organs in the body. Our main goal is to better understand the mechanisms controlling damage in infected lung alveolar epithelial cells. We assumed that lung alveolar cells infected with *A. fumigatus* undergo a specific and pre- programmed molecular response. Two approaches were used to analyze the response of infected cells: A) Kinome analysis, identification of specific phospho-proteins using a panel of 36 mono- specified antibodies. B) Transcriptional analysis using Affymetrix arrays. A549 lung epithelial cells were infected with *A. fumigatus* conidia or culture filtrate and compared to uninfected cells. Kinome analysis revealed five main proteins, Adducin gamma, PKR, Erk1/2, JNK and MEK1/2 which showed differences in phosphorylation patterns between infected and uninfected cells. These results suggest that the infected cells mount a strong JNK/MAPK pathway stress response to *A. fumigatus* infection. Interestingly, adducin, a cytoskeletal protein, was phoshphorylated more intensively in response to conidia than to culture filtrate, whereas PKR, which plays a role in mediating the activation of the MAPK pathways, was phoshphorylated only in response to culture filtrate. Affymetrix transcriptional analysis revealed 388 genes differentially expressed at least 2 fold ($p < 0.05$) in response to infection by germinated conidia, and 478 genes in response to culture filtrate. Interestingly, the response to germinated conidia was characterized by strong (>5 fold) upregulation of 35 genes involved in cell death, immune response, cytokine activity (especially IL8) and response to wounding. A significant number of these genes is controlled by the transcription factor NFk-B. In contrast, the response of A549 cells to culture filtrate was mainly characterized by the down regulation of 277 genes involved in metabolism and biosynthesis. Our results demonstrate that A549 lung cells respond differentially to infection with *A. fumigatus* conidia or culture filtrate. We propose that during early conidial infection, infected cells mount a vigorous protective response. In contrast, during late infection, the accumulation of secreted culture filtrate elicits a marked inhibition of cellular metabolism. These results help clarify the progression of cellular infection by *A. fumigatus* at the molecular level, and suggest novel ways to interfere with this destructive process.

184. **Metabolic network-driven analysis of genome-wide transcription data from *Aspergillus nidulans.*** Helga Moreira David[1], Gerald Hofmann[2], Ana Paula Oliveira[2], Hanne Jarmer[3], and Jens Nielsen[2]. [1]Fluxome Sciences A/S, Diplomvej 378, 2800 Lyngby, Denmark [2] Center for Microbial Biotechnology, Technical University of Denmark, 2800 Lyngby, Denmark [3] Center for Biological Sequence Analysis, Technical University of Denmark, 2800 Lyngby, Denmark

Aspergillus nidulans is a model organism for aspergilli, an important group of filamentous fungi that encompasses human and plant pathogens, as well as industrial cell factories. Aspergilli have a highly diversified metabolism and, because of their medical,

agricultural and biotechnological importance, it is valuable to understand how their metabolism is regulated. We therefore performed genome-wide transcription analysis of A. nidulans grown on glucose, glycerol, and ethanol with the objective of identifying global regulatory structures. We furthermore reconstructed the complete metabolic network of this organism, which resulted in linking 666 genes to metabolic functions, as well as assigning metabolic roles to 472 genes that were previously uncharacterized. Through combination of the reconstructed metabolic network and the transcription data, we identified subnetwork structures that pointed to coordinated regulation of genes involved in many different parts of the metabolism. Thus, for a shift from glucose to ethanol, we identified a coordinated regulation of the complete pathway for oxidation of ethanol, as well as up-regulation of gluconeogenesis and down-regulation of glycolysis and the Pentose Phosphate (PP) pathway. Furthermore, upon a change in the carbon source from glucose to ethanol, the cells shift from using the PP pathway as the major source of NADPH for biosynthesis to use of the malic enzyme. Our analysis indicated that some of the genes are regulated by common transcription factors, making it possible to establish new putative links between known transcription factors and genes, through clustering.

185. T**he *Leptosphaeria maculans* genome initiative.** M.-H. Balesdent[1], B. J. Howlett[2], P. Wincker [3], J. Amselem[1,5], A. Cozijnsen[2], I. Fudal[1], L. Gout[1,4], M. Meyer [1], A. Stachowiak [6], A. Van De Wouw [2], T. Rouxel[1]. [1]INRA-PMDV, F-78026 Versailles,France; [2]School of Botany, Uni-Melbourne, Parkville, VIC 3010, Australia; [3]Genoscope, 91057 Evry Cedex, France ; [4]Protection des Plantes, INA-PG, 78850 Thiverval-Grignon, France;

[5]INRA-URGI, 91000 Evry, France; [6]IGR, Poznan, Poland

Genoscope (National Sequencing Centre), the French sequencing agency is involved in large-scale sequencing of the genome of the Dothideomycete *Leptosphaeria maculans*. Previous collaborative projects between INRA Versailles and Genoscope consisted in precise sequencing, assembly and finishing of a 1.1 Mb genomic region in isolate v23.1.3. These data enabled characterization of the first known retrotransposons and avirulence genes in *L. maculans*. These analyses also showed that the genome comprised regions of long A+T-rich regions reminiscent of heterochromatin, alternating with G+C-equilibrated, gene-rich regions. The goal of the genome initiative is to release an annotated assembly with 12-x genome coverage for *L. maculans* isolate v23.1.3. Additional sequencing of BAC-ends for physical mapping and ESTs from mycelia grown under three different conditions is underway. The first draft 5-x coverage indicated a high content of repeats in the genome. The first 12-x assembly was released Nov. 15, 2006, and comprised of 1277 super contigs of which 142 are more than 100 kb (mean : 39.78 kb; max : 866.65 kb). From these data, a bigger- than-expected genome size of ca. 50 Mb is hypothesised and a ca; 30% of repeats in the genome is suggested. First analysis from the final assembly (expected in January 2007) will be presented. A. Stachowiak was funded by Marie-Curie Fellowship HPMT-CT-2001-00395 ‘FUNGENE’

186. **The cyclic AMP induced gene expression of *Magnaporthe grisea* during appressoria formation.** Yeon Yee Oh, Shaowu Meng, Douglas E. Brown, Sean Coughlan[1], Thomas K. Mitchell, Ralph A. Dean Fungal Genomics Lab, North Carolina State University, Raleigh NC. [1]Agilent Technologies, Inc. Wilmington DE.

The cyclic AMP signaling pathway modulates appressoria development in rice blast fungus, *Magnaporthe grisea*. To identify the cAMP induced cellular processes for appressoria induction, we compared global gene expression patterns in cAMP elaborating appressoria verse germinating spores on non inductive hydrophilic surfaces. After 9 hours incubation, RNA was extracted from tissues of each condition and microarray experiments were performed using the *M. grisea* v2.0 oligonucleotide array, created in collaboration with Agilent Technology. 8.6 % of *M. grisea* genes were found to be differentially expressed by cAMP treatments. This included some previously known pathogenecity factors. Functional analysis suggests that melanin biosynthesis, secondary metabolism, protein degradation, protein synthesis and lipid degradation are downstream of cAMP signaling pathway and are necessary for appressoria morphogenesis. This was verified by gene knock out followed by phenotype analysis. We will present the results of gene expression studies, classification of differentially expressed genes and functional characterization of selected genes.

187. **Natural history and evolutionary principles of gene duplication in fungi.** Ilan Wapinski, Avi Pfeffer, Nir Friedman, Aviv Regev. Broad Institute, MIT, Cambridge, MA USA e-mail: ilan@eecs.harvard.edu

Gene duplication and loss is a powerful source of functional innovation. However, the general principles that govern this process are still largely unknown. With the growing number of sequenced genomes, it is now possible to examine these events in a comprehensive and unbiased manner. Here, we develop a novel procedure that resolves the evolutionary history of all genes in a large group of species. We apply our procedure to seventeen fungal genomes to create a genome- wide catalog of gene trees that determine precise orthology and paralogy relations across these species. We show that gene duplication and loss is highly constrained by the functional properties and interacting partners of genes. In particular, stress-related genes exhibit many duplications and losses while growth-related genes show selection against such changes. This dichotomy is relaxed following whole-genome duplication. Duplicated genes rarely diverge with respect to biochemical function, but typically diverge with respect to regulatory control. Surprisingly, paralogous modules of genes rarely arise, even following whole-genome duplication.

Rather, gene duplication drives the modularization of functional networks through specialization, thereby disentangling cellular systems.

188. **The effector secretome of *Phytophthora infestans*: Structure and function**. Sophien Kamoun [1], Joe Win [1], William Morgan [1], Sang-Keun Oh [1], Carolyn Young [1], Rays Jiang [2, 3], Francine Govers [3], Michael C. Zody [2], Chad Nusbaum [2]. [1] Department of Plant Pathology. The Ohio State University, OARDC, Wooster, OH, USA. [2] The Broad Institute of MIT and Harvard, Cambridge, MA, USA. [3] Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, The Netherlands.

The oomycete *Phytophthora infestans* causes late blight of potato and tomato and is arguably the most destructive pathogen of solanaceous crops. *P. infestans* establishes parasitic colonization of plants by modulating host cell defenses through an array of disease effector proteins. The biology of *P. infestans* effectors is poorly understood, but tremendous progress has been made recently. Two classes of effectors target distinct sites in the host plant: apoplastic effectors are secreted into the plant extracellular space, while cytoplasmic effectors are translocated inside the plant cell, where they target different subcellular compartments. Of particular interest are the RXLR effectors that are characterized by a conserved motif following the signal peptide. The RXLR domain is functionally interchangeable with a malaria host targeting domain and appears to function in delivery into host cells. The recent completion of the genome sequence of *P. infestans* enables genome-wide cataloguing of the effector secretome. Using computational analyses, we identified up to 700 candidate RXLR effector genes. These were frequently organized in clusters of paralogous genes, many of which exhibit hallmarks of positive selection probably as a result of a coevolutionary arms race with host factors. Predictably, effector genes are typically expressed and often up-regulated during infection. We also utilized the discovered RXLR effectors in high-throughput *in planta* expression assays to screen for alteration of plant defense response and gain an insight into their function.

189. Withdrawn

190. **Withdrawn**

191. **Proteome analysis of the response of *Aspergillus fumigatus* to iron limitation.** André D. Schmidt[1], Olaf Kniemeyer[1], Hubertus Haas[2] and Axel A. Brakhage[1]. [1]Leibniz Institute for Natural Product Research and Infection Biology (HKI) / Friedrich-Schiller-University Jena, Germany [2]Division of Molecular Biology/Biocenter, Innsbruck Medical University, Austria

The acquisition of iron is known to be an essential step in any microbial infection process due to iron-limiting conditions in the human host. This iron limitation is caused by high-affinity iron-binding proteins like transferrin or lactoferrin in the host. Since iron plays an essential role in key metabolic processes like DNA synthesis, oxidative phosphorylation or electron transport *A. fumigatus* has to overcome the iron deficiency by the synthesis of siderophores, which chelate iron. It was shown that an *A. fumigatus* strain unable to synthesize siderophores was attenuated in virulence in a murine infection model. To understand the cellular processes, induced by iron starvation, we analysed the proteome of *A. fumigatus* strain ATCC 46645 grown under iron-deficiency conditions. Under iron depletion, proteins involved in siderophore biosynthesis are upregulated, e.g. L-ornithine N^5-oxygenase (SidA), and iron cluster-containing proteins as aconitase or 3-isopropylmalate dehydratase are down-regulated. In addition, proteins involved in the heme biosynthesis are less abundant under iron-deficiency. Further proteins analysed under different non- linear pH-scales will be presented and their putative role will be discussed.

192. **Comparative Genomics of plant pathogenic Fusarium species.** Li-Jun Ma[1], Won-Bo Shim[2], Manfred Grabherr[1], Michael Koehrsen[1], Reinhard Engels[1], Sinead O'Leary[1], Matter Pearson[1], Sarah Young[1], David DeCaprio[1], Chinnappa Kodira[1], James Galagan[1], Seogchan Kang[3], Charles Woloshuk[4], Harold Corby Kistler[5], Bruce Birren[1]. [1]Broad Institute of MIT and Harvard. [2]Texas A&M University. [3]Penn State University. [4]Purdue University. [5]University of Minnesota.

The genus Fusarium collectively represents the most important group of fungal plant pathogens, causing various diseases on nearly every economically important plant species. Of equal concern is the health hazard posed to humans and livestock by the plethora of *Fusarium mycotoxins*. Our goal is to utilize the power of comparative genomics to improve gene annotation, to identify functional non-coding elements and to study the evolution and pathogenicity of this group of economicly important species. *F. oxysporum* and *F. verticillioides* have been selected as sequence targets to facilitate the comparative studies with the existing *F. graminearum* genome. Sequencing, assembly and automated annotation of *F. verticillioides* is complete and sequencing and assembling for *F. oxysporum* is ongoing. The released *F. verticillioides* assembly contains 41.7Mb with 14,206 predicted proteins. More than 99% of the assembly was anchored to the genetic map. A newly constructed optical map for *F. oxysporum* spans more than 60Mb, which is much bigger than either *F. graminearum* or *F. verticillioides*. Preliminary analysis of the sequence indicates that the increased genome size is mainly caused by large segmental duplications and the existence of many transposable elements. One of the focal points of our comparative analysis is to identify gene clusters associated with mycotoxin production and tracing their evolutionary history. We will report our recent progress and discuss the impact of comparative

genomics on our understanding of genome dynamics, pathogenicity and mycotoxin production in these genomes. Funding for Fusarium comparative project was provided by USDA/NSF microbial genome sequencing program.

193. **Aspergillus Comparative Database: a web-based tool for comparative analysis.** Vinita Joardar, Jonathan Crabtree, Rama Maiti, Natalie Fedorova, Paolo Amedeo, Samuel Angiuoli, William Nierman, Owen R. White, and Jennifer R. Wortman. The Institute for Genomic Research, Rockville, MD, USA. vinita@tigr.org

Comparative genome analysis in the genus Aspergillus has been facilitated by the availability of genome sequences for multiple species. Ortholog clusters were computed based on the mutual best blastP hits between the Aspergillus proteomes. Syntenic blocks were identified by searching for collinear orthologs, with allowances for gaps and rearrangements, along the full chromosomes and/or supercontigs. The results of the genome and proteome level computes for the Aspergillus genomes were stored in the Aspergillus Comparative Database (asp), a chado relational database. Sybil, a web-based software package developed at TIGR, was used for visualization and analysis of comparative genomics data. Sybil uses a graphical user interface to present and navigate the information stored in asp. The interactive graphical displays allow the user to navigate from global genome views down to specific protein reports. Protein cluster reports, lists of singletons, comparative sequence displays and publication-quality figures can be customized based on user specifications. The Sybil package also leverages the comparative data for annotation improvement. We present an overview of the Sybil package applied to the comparative analysis of aspergilli (http://www.tigr.org/sybil/asp/index.html). The Sybil visualization software is freely available for download from http://sybil.sf.net

194. Using comparative genomics to identify virulence characteristics in pathogenic yeast. David Fitzpatrick and Geraldine Butler. School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Ireland.

Due to their increasing clinical importance, nine *Candida* and closely related species have been sequenced. The wealth of genomic data now available, allows us to investigate the differences that account for increased pathogenicity in particular *Candida* species. In order to identify specific metabolic activity associated with pathogenesis, we have assigned KEGG orthology identifiers to all available *Candida* genome data, using a bidirectional best BLAST strategy against the manually curated KEGG GENES database. All annotated *Candida* data has subsequently been mapped onto reference metabolic pathways from the KEGG PATHWAY database. Using the information gleaned from our metabolic pathway analysis we have attempted to correlate species-specific components with virulence, by noting which are specific to pathogenic *Candida* such as *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis* but absent in closely related non-pathogenic species such as *Debaryomyces hansenii* and *Lodderomyces elongisporus*. Pathways that are present in numerous pathogenic *Candia* species are of special interest as they have the potential to yield broad-spectrum targets for novel antifungal drugs. To help visualize genotypic and metabolic differences between species, we have also developed the *Candida* Gene Order Browser, an online tool that displays the syntenic context of genes from all available *Candida* genomes.

195. **The grass endophyte *Epichloe festucae*: genome, unigenes, and gene expression.** U. Hesse, P. Maynard, S. Macmil, G. Wiley, K. Andreeva, W. E. Beech, C.D. Van Horn, V.C. Bumgardner, V. Gopal- Puram, J. Wiseman, J. Webb, L. Gill, S. Alluri, E. Arnaoudova, M.L. Farman, J. W. Jaromczyk, B.A. Roe, C.L. Schardl. University of Kentucky, Plant Pathology Dept.

The ascomycete, *Epichloe festucae*, is a model endophyte that 1) switches between mutualistic and antagonistic states, 2) is seed transmissible, 3) has a sexual state amenable to genetic analysis, and 4) is rich in bioprotective alkaloids. This fungus grows systemically and intercellularily throughout the life of its host plant. On each reproductive tiller the fungus either infects benignly and transmits clonally in seeds, or produces its sexual state (stroma) and chokes inflorescence development. The *E. festucae* genome was estimated at 29 Mb in 6 chromosomes. The genome sequence was assembled from cloned insert end reads (4.2 x coverage) and preassembled pyrosequencing reads (454-sequencing: 20 x raw, 1.7 x assembled), giving 3967 supercontigs, of which 1004 larger than 2 kb covered ca 92% of the genome. Gene prediction (FGENESH) identified 9912 putative genes. We sequenced 25,000 ESTs from each of two normalized libraries — one of choked inflorescences, the other of benignly infected inflorescences — yielding 5077 *E. festucae* unigenes annotated by BLAST and InterPro. Additionally, ca 700,000 pyrosequencing reads were obtained from non-normalized cDNA derived from four biological replicates each of asymptomatic and choked inflorescences. Of these, 118,865 were identified in the *E. festucae* genome sequence, 23,046 in the unigene set, and 21,703 in the predicted gene set, allowing analysis of gene expression and *in symbio* regulation. Sequences are BLASTable at http://www.genome.ou.edu/blast/ef_blastall.html, Sequence data and annotations are stored in a MySQL database, soon to be published in GBrowse.

196. **Analysis of *TOX2*, the HC-toxin biosynthetic gene cluster in *Cochliobolus carbonum*.** Kouhei Ohtani, John S. Scott-Craig, and Jonathan D. Walton. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA.

HC-toxin is a cyclic tetrapeptide host-selective toxin produced by the filamentous fungus *Cochliobolus carbonum*, a pathogen of maize. HC-toxin production in *C. carbonum* is controlled by a single locus, *Tox2*, which is composed of at least seven duplicated and coregulated genes involved in HC-toxin biosynthesis, export, and regulation. The *Tox2* locus was earlier partially characterized by mapping, sequencing, and gene disruption but we still didn't know its overall structure nor how many additional genes are required for HC-toxin biosynthesis. To understand more thoroughly the structure of *Tox2* and to find new genes involved in HC-toxin biosynthesis, we constructed a bacterial artificial chromosome (BAC) library from a *C. carbonum* toxin-producing strain and screened it with known *Tox2* genes. More than six BAC clones were identified and shotgun sequenced to at least 4x coverage. To date we have identified several new putative *Tox2* genes. One encodes an alpha subunit of fatty acid synthase, which is predicted to complex with the product of *TOXC* (encoding a beta subunit of fatty acid synthase) to synthesize the backbone of Aeo (2-amino-9,10-epoxi-8-oxodecanoic acid). Other new genes encode three P450s and an isoamyl alcohol oxidase. Like the known *Tox2* genes, all of these new genes are absent in toxin non-producing isolates of *C. carbonum*. All have plausible roles in HC-toxin biosynthesis, which is being tested by targeted gene disruption.

197. **The Genome Sequence of the Brown Rot Fungus *Postia placenta***. Jean F. Challacombe[1], Diego Martinez[1], Thomas S. Brettin[1], Susan Lucas[2], Paul Richardson[2], Eddy Rubin[2], and Dan Cullen[3]. [1]Los Alamos National Laboratory and DOE Joint Genome Institute, Los Alamos, NM. [2]DOE Joint Genome Institute, Walnut Creek, CA. [3] USDA, Forest Products Laboratory, Madison, WI.

The brown rot fungi, a group of filamentous basidiomycetes, rapidly depolymerize the cellulose in wood without significant lignin removal. This type of decay differs from white rot fungi, such as *Phanerochaete chrysosporium*, which simultaneously degrade lignin and cellulose. Both white and brown rot fungi are common inhabitants of forest litter where they play an important role in carbon cycling. Brown rot fungi are most commonly responsible for the destructive decay of wood in buildings and other structures, and it has been estimated that 10% of the U.S. timber harvest decays in service each year. Irrespective of their economic importance, the mechanism of brown rot wood decay is poorly understood. To better understand the biology of this type of wood degradation, the Department of Energy's Joint Genome Institute has sequenced the entire 33Mb genome of the model brown rot, *Postia placenta*. Using genomic DNA from dikaryotic strain MAD-698, the JGI generated 571,000 reads that assembled into 1243 haplotype scaffolds. Our investigation into the genome will provide an invaluable mechanistic perspective on cellulose depolymerization, and the unique haplotype assembly will allow us to gain insights into the complexities of genome evolution.

198. **Withdrawn**

199. **Whole genome sequencing of the fungal plant pathogens *Botrytis cinerea* and *Sclerotinia sclerotiorum***. Sabine Fillinger[1],François Artiguenave[2], Alain Billault[3], Mathias Choquer[1], Arnaud Couloux[2], Christina Cuomo[4], David DeCaprio[4], Martin Dickman[5], Elisabeth Fournier[1], James Galagan[4], Corinne Giraud[1], Chinnappa Kodira[4], Linda Kohn[6], Fabrice Legeai[2], Caroline Levis[1], Evan Mauceli[4], Cyril Pommier[2], Jean-Marc Pradier[1], Emmanuel Quevillon[2,8], Jeffrey Rollins, Béatrice Ségurens[3], Adeline Simon[1], Muriel Viaud[1], Jean Weissenbach[3], Patrick Wincker[3], and Marc-Henri Lebrun[8]. [1]UMR BIOGER, INRA, Versailles, France; [2]URGI,INRA, Evry, France;[3]Génoscope- CNS, Evry, France; [4] The Broad Institute of Genome Research, Cambridge MA, USA; [5] Inst. for Plant Genomics and Biotech, College Station TX, USA; [6] University of Toronto, Mississauga, Canada; [7]Department of Plant Pathology,University of Florida, Gainesville, FL, USA [8] Plant and Fungal Physiology, CNRS-BayerCropScience, Lyon, France

Botrytis cinerea and *Sclerotinia sclerotiorum* are destructive polyphageous pathogens of many economically important crops provoking grey and white mould respectively. Both fungi are leotiomycetes. In 2005, the Broad Institute released the assembly of a 4-5 x genomic sequence from *B. cinerea* strain B05-10 (TMRI/Syngenta) as well as the 7-8 x genomic sequence of *S. sclerotiorum*. At the same time, the French national sequencing center (Genoscope) started the genome sequencing of grapevine and two of its pathogens: Stolbur phytoplasm and *B. cinerea* strain T4. In addition to WGS 50.000-60.000 ESTs have been sequenced for both species. Automatic gene prediction with *ab initio* and similarity softwares has been applied to the three genomes suggesting 14.000 to 16.000 potential gene calls for both species. A manual annotation process involving an international consortium of 20 research groups will validate and correct the data. Comparative analyses of both *B. cinerea* strains on one hand and *B.cinerea > vs. S. sclerotiorum* on the other hand revealed a common set of 8400 gene calls, 1200 of which are absent from other fungal genomes. In addition both species present a high degree of sequence similarity (76 % average sequence identity in orthologous proteins), and of synteny at the contig level. *S. sclerotiorum* has twice as much repetitive elements (7.7 %) than *B. cinerea* (3 - 4 %) with an enrichment of all transposable elements and tandem repeats. RIPping also occurred at higher levels in *S. sclerotiorum*. The comparison between *B. cinerea* and *S. sclerotiorum* offers the first opportunity to compare the genomes of two closely related necrotrophic plant pathogens helping understanding evolutionary trends that have shaped their genomes.

200. **Global Gene Expression Profiles of *Phytophthora ramorum* strain Pr102 in response to plant host and tissue differentiation**. Caroline Press and Niklaus Grunwald, USDA, 3420 NW Orchard Ave., Corvallis, OR 97330. pressc@science.oregonstate.edu

The release of the draft genome sequence of *P. ramorum strain Pr102*, enabled the construction of an oligonucleotide microarray of the entire genome of Pr102. The array contains 344,680 features (oligos) that represent the transcriptome of Pr102. *P. ramorum* RNA was extracted from mycelium and sporangia and used to compare gene expression across tissue types and in the presence of the host (*Rhododendron* sp.). The purpose of the experiment was to identify genes where expression was responsive to tissue types and upon exposure to the host plant for further study. Gene expression studies were performed using a Nimblegen microarray. Genes were determined to be differentially expressed between tissue types if they were statistically significant (P = 0.05 after FDR) and resulted in a greater than 20-fold change in gene expression. In the comparison between mycelia and sporangial tissues, 263 genes demonstrated a greater than 20 fold change in gene expression. Of those genes, 52 genes were significantly downregulated in sporangia as compared to mycelium and 214 genes were significantly upregulated in sporangia as compared to mycelium. Several of the differentially expressed genes appear to be of the same type as those in a similar study in *Phytophthora infestans* by Kim and Judelson (2003) and include genes involved in cell wall restructuring, cell division and signaling functions. Not surprisingly, several genes involved in energy production, electron transport chains and growth are reduced in sporangia. 50% of the genes with differential expression have not yet been classified as to function in the current annotation. Further experimentation and analysis is ongoing.

201. **Computational Resources for Fungal Genomics.** Gangman Yi[1], Jaehee Jung[1], Serenella A. Sukno[2], and Michael R. Thon[1,2]. Texas A&M University, [1]Department of Computer Science, and [2]Department of Plant Pathology & Microbiology, College Station, TX USA

As the number of sequenced fungal genomes continues to grow, there is an increasing need for effective algorithms for whole genome analyses. We present two applications that are aimed at improving our knowledge of gene function and genome evolution. First, we present C-Hunter, an algorithm and software application designed to identify clusters of functionally related genes in genomes. C-Hunter utilizes functional categories defined in graph-based vocabularies such as the Gene Ontology (GO). Clusters identified in this manner need only have a common function and are not constrained by gene expression patterns or other properties. C-Hunter source code and example report files are freely available from our web site. We also present AAPFC (Automated Annotation of Protein Functional Class). AAPFC addresses the need for high quality, automated functional annotation of proteins in fungal genomes. The method utilizes data mining and statistical pattern recognition techniques to learn models for assigning Gene Ontology functional categories to unannotated proteins. In its current implementation, AAPFC is trained using fungal GO-annotated proteins obtained from the UniProt database. The AAPFC web site provides users with GO annotations and protein features in graphical and textual formats. Users can annotate small sets of proteins via a web interface and large sets of proteins by contacting the authors.

202. **Transcriptional patterns in the extramatrical mycelium of the ectomycorrhizal fungus *Paxillus involotus* in response to various sources of nitrogen.** Derek P. Wright, Dag Ahren, Anders Tunlid & Tomas Johansson Microbial Ecology, Ecology Building, Lund University, Sweden

The positive effects of ectomycorrhizal (ECM) fungi on plant nutrition have traditionally been attributed to the quantitative effect of the extramatrical mycelium on uptake of dissolved nutrients, such as nitrogen. In forest soils nitrogen is present either in inorganic (mainly as ammonium) or organic (amino acids, peptides and proteins) forms and nitrogen mobilization by hyphae from soil is directly linked to hyphae uptake capacities. Transcriptional programs for assimilation of various sources of inorganic and organic forms of nitrogen were investigated in the ECM association between *Paxillus involotus* and birch. Nitrogen sources of various degrees of complexity, ammonium phosphate, ammonium sulphate, glutamine, chitin, and bovine serum albumin were provided as patches in peat microcosms for fungal in growth. After fungal establishment in these patches total RNA was isolated and used for global transcriptional analyses. We used a custom cDNA microarray containing approx. 4,900 gene representatives. By bioinformatic means and using EST sequence information we could among those identify 462 reporters representing putative enzymes. From a metabolic reconstruction of these 462 enzymes we found them potentially involved in 901 enzymatic reactions and 173 metabolic pathways. By combining this information with transcriptional data we could observe major shifts in a number of metabolic pathways as responses to the nitrogen source provided.

203. **Comparative Genomic Hybridization in the Genus Neurospora: Assessing the Ability to Recover Evolutionary Relationships.** Luz B. Gilbert*, Lee Chae, Takao Kasuga, Louise Glass, and John W. Taylor Department of Plant and Microbial Biology, UC Berkeley, *lgilbert@berkeley.edu

Comparative Genomic Hybridization (CGH) using DNA microarrays is becoming a popular method of determining phylogenetic relationships among individuals and even species. Although researchers praise the large-scale genome information output as an advantage of this technology for use in phylogenetic analysis, few have questioned the reliability of CGH to correctly determine

evolutionary relationships. With the tools at my disposal for the filamentous fungus *Neurospora*: a genome sequence, a DNA microarray, and a well supported phylogeny for this genus, I had the ability to rigorously address the utility of CGH for phylogenetic studies using both experimental and simulated data. The results suggest constructing phylogenetic trees from this type of data requires careful consideration of which tree-building method is used and which taxon is printed on the array.

204. **Analysis of the *Magnaporthe grisea* secretome by liquid IEF fractionation and mass spectrometry.** Gerardo Gutierrez-Sanchez, Punit Shah, James Atwood III, Denise Lennon, Estee-Lina Tran, Peter Albersheim, Alan Darvill, Ron Orlando and Sheng-Cheng Wu. Complex Carbohydrate Research Center,University of Georgia, 315 Riverbend Road, Athens, GA 30602-4712, USA.

Magnaporthe grisea, a fungal plant pathogen, is a major threat to rice production globally. The *M. grisea* genome encodes many secreted proteins and in response to various growth conditions it secretes extracellular proteins (ECPs) which play indispensable roles in host pathogen interactions. For many years, plant pathologists have concentrated on elucidating the relation between environmental factors and field development of the disease, developing fungicides, or breeding resistant cultivars. However, molecular basis of the resistance mechanisms in rice plants against *M. grisea* or of host specificity for *M. grisea* has not been clearly elucidated. A more extensive catalog of the secretome would therefore represent a valuable resource to elucidate processes such as pathogenesis and disease resistance. The aim of this study is to gain new insights into the dynamics and complexity of the proteins that are secreted by both *Magnaporthe grisea* and its rice host (*Oryza sativa* L.) during infection. To date, most of the proteomics studies in plant pathogenic fungi have been limited to 2-D gel analysis. Here we present a simple and effective "non-gel"-based fractionation method to separate the secreted proteins from *M. grisea* and rice plant, followed by LC-MS/MS analysis for protein identification and statistical validation with the PROVALT algorithm."

205. **Preliminary GO annotation of a blast fungus *Magnaporthe grisea*.** Shaowu Meng, Douglas E. Brown, Thomas K. Mitchell, Ralph Dean*. Center for Integrated Fungal Research, North Carolina State University, Campus Box 7251, 851 Main Campus Drive, Raleigh, NC 27695

The *Magnaporthe grisea* genome assembly Version 5 was released in the spring of 2006 along with automatic annotations. The next step is a curated community annotation effort. Gene ontology (GO) based systems are evolving into a reliable and rapid means of assigning annotations. Here, we report our preliminary GO annotation of *M. grisea* genome. This was done in two steps. Firstly, we identified orthologs between *M. grisea* and *Saccharomyces cerevisiae* through cataloging reciprocal best hits between translated ORFs of the two organisms using blastp. The cataloging was iteratively performed on the remaining un-annotated genes until the last reciprocal best hit was found. The result was 4,464 unique *M. grisea* ORFs annotated using 2,581 unique *S. cerevisiae* GO terms totally at 26,966 times. Next, for those *M. grisea* ORFs which did not have orthologs in the *S. cerevisiae* database, we applied the cataloging process using the GO proteins databases, which included multiple organisms. In this step, 1,760 unique *M. grisea* ORFs were annotated using 1,263 unique GO terms totally at 5,381 times. The GO annotation of *M. grisea* covered 50.9% of predicted genes. Our GO annotation provides a base for further functional genomics analysis of *M. grisea*, and for assigning newly created terms from the PAMGO community that are specific to plant pathogens.
*Corresponding author.

206. **Conserved Synteny in *Trichoderma ressei* with Related Filamentous Ascomycetes.** Diego Martinez[1,2], Mary Anne Nelson[2] and Margaret Werner-Washburne[2]. [1]Los Alamos National Laboratory/Joint Genome Institute, Los Alamos, NM 87018
[2]Department of Biology, University of New Mexico, Albuquerque, NM 87131

The current pace of whole genome sequencing has made it almost trivial to produce nearly complete fungal sequences. This now gives us the ability to perform whole genome comparisons of fungi to investigate biological questions and genome evolution. To this end, syntenic regions between *Trichoderma reesei* and four other filamentous fungi (*Fusarium graminearum, Neurospora crassa, Magnaporthe grisea and Aspergillus nidulans*) were identified. An algorithm was designed that takes homologs from two fungi and places them side by side, optimizing homolog density, and minimizing gaps between homologs and the number of homologs in a syntenic region. As would be expected, the percent coverage of synteny between *T. reesei* and the other four ascomycetes declines with time since the last common ancestor, with *F. graminearum* having the highest synteny and *A. nidulans* the lowest. This approach enabled the identification of regions in the *T. reesei* genome that were conserved in multiple genomes. Finally, for genes within these highly conserved regions, GO terms and Enzyme Commission codes were assigned. These results were used to assess the possibility that there is selective pressure to force genes that are in the same biochemical pathway, have similar function or are involved in similar processes to maintain proximity.

207. **Comparative proteomics of the in vitro and in planta secretomes of *Fusarium graminearum*.** Janet M. Paper, John S. Scott-Craig and Jonathan D. Walton. DOE-Plant Research Laboratory, Michigan State University, E. Lansing MI 48824

F. graminearum (Fg) is the cause of head blight and stalk rot of wheat and maize. Its sequenced genome is predicted to encode ~12,000 proteins. Secreted fungal proteins have several known functions related to pathogenesis, including phytotoxicity, degradation of cell wall polymers (penetration, ramification, and nutrition), and inhibition of plant defense proteins. We are using peptide mass fingerprinting to analyze the secretome of Fg in vitro and in planta. Secreted proteins from Fg grown in vitro on different media or extracted from infected wheat heads by vacuum infiltration were digested en masse with trypsin and analyzed by LC-MS/MS. From culture filtrates of Fg grown on sucrose, carrot or maize cell walls, collagen, xylan, pectin, or oat bran, ~230 proteins could be identified with high statistical probability. Because many secreted proteins are catabolite-repressed and/or substrate-induced, the spectrum of proteins was influenced by the medium, e.g., collagen induced the most proteases and sucrose yielded the fewest hydrolases. Despite the preponderance of host proteins in infected wheat heads, we could identify 120 fungal proteins. Many of these (25) were predicted to interact with host cell walls (e.g., hydrolases, sugar oxidases, cellulose-binding proteins, acetylxylan esterases). Some (42) in planta proteins were not found any of the in vitro analyses. About 27% of the in planta proteins were of unknown function or predicted to be non-enzymatic. Although >85% of the in vitro proteins were predicted to have signal peptides, only 61% of the in planta proteins were. Among the many predicted non-secreted proteins found in planta were 13 housekeeping enzymes including enolase, triose phosphate isomerase, phosphoglucomutase, calmodulin, aconitase, and malate dehydrogenase. The presence of these proteins in the in planta secretome might be due to fungal lysis in planta, but several of them have been reported to be immunogens secreted by animal pathogenic fungi.

208. **Simple Sequence Repeats in *Neurospora crassa* : distribution, polymorphism, and marker potential.** Tae-Sung Kim[1], James Booth[2], Hugh G. Gauch, Jr.[3], Qi Sun[4], Jongsun Park[5], Kwangwon Lee[1]. [1]Department of Plant Pathology, [2]Department of Biological Statistics and Computational Biology, [3]Department of Crop and Soil Sciences, Cornell University, Ithaca, NY 14850 USA [4]Cornell Theory Center, Ithaca NY 14850 USA [5]Fungal Bioinformatics Laboratory and Department of Agricultural Biotechnology Seoul National University, Seoul 151-921, Korea.

Simple sequence repeats (SSRs) have been successfully used as molecular markers for various genetic studies in eukaryotic systems. Despite the growing number of sequenced genomes, a thorough statistical examination on the distribution of the SSRs and experimental verification of the usefulness of SSRs in filamentous fungi for genetic and genomics studies are lacking. We identified and characterized the 2749 SSRs of 963 SSR types in the whole genome of the model fungal system *Neurospora crassa*. The tri-nucleotide (nt) repeats were the most common SSRs in *N. crassa*. The distribution of the tri-nt SSRs was significantly biased in exons. We further characterized the distribution of 18 abundant SSR types, which account for 71% of total SSRs in the *N. crassa* genome. We tested whether the occurrence rate of SSRs is random across chromosomes using Poisson log linear model. Based on our analyses, we concluded that there are systematic differences in SSR occurrence rates between chromosomes as well as between SSR types. We attempted to explain the non-randomness of the SSR occurrence between the chromosomes using factors such as chromosome size and gene density. We also characterized the size variation of SSRs among accessions using Polymorphic Index Content (PIC). We characterized the PIC scores of abundant SSR types using randomly selected 162 SSR loci in seven *N. crassa* accessions. Finally, utilizing the polymorphic SSRs, we built three linkage maps from three intra-species specific populations. The genetic orders of SSR markers along the chromosomes from the three linkage maps were highly consistent with the position in the physical map suggesting that the genetic architectures of the six natural accessions are similar to each others.

209. **Annotation and analysis of the genome of *Phycomyces blakesleeanus*, a model photoresponsive zygomycete.** Alan Kuo[1*], Asaf Salamov[1], Jasmyn Pangilinan[1], Erika Lindquist[1], Harris Shapiro[1], Scott Baker[2], Luis Corrochano[3], and Igor Grigoriev[1]. [1]DOE Joint Genome Institute, Walnut Creek, CA, USA. [2]Pacific Northwest National Laboratory, Richland, WA, USA. [3]Departamento de Genetica, Universidad de Sevilla, Spain. [*]akuo@lbl.gov

Light induces in *P. blakesleeanus* multiple developmental and biochemical responses (sporangiophore growth and development, beta-carotene synthesis). *P. blakesleeanus* is an intensively studied, experimentally tractable model organism, and whole-genome analysis is expected to further elucidate the signaling pathways underlying its photoregulation. To this end the genome was sequenced to 7.49X depth and assembled into 475 scaffolds totaling 56Mbp, and 47847 ESTs were assembled from cDNAs of light and dark cultures. We combined into a single annotation pipeline a variety of gene modeling methods (homology-based, EST-based, and *ab initio*), and predicted 14792 protein-coding genes. Many of these gene predictions are supported by homology in nr (68%), by Pfam domains (44%), or by ESTs (35%). We next assigned GO terms to 41% of the proteins and EC numbers to 16%. We then distributed these annotations to the Phycomyces consortium, along with tools to curate them manually. We expect that the annotation will provide a solid platform for expression analysis. In addition to its value as a model organism, *P. blakesleeanus* is the second zygomycete with a sequenced genome, after the related *Rhizopus oryzae*. We therefore will present preliminary results of comparative analysis between the two zygomycetes. LBNL-62091 ABS This work was performed under the auspices of the US Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48, Lawrence Berkeley National Laboratory under contract No. DE-AC02-05CH11231 and Los Alamos National Laboratory under contract No. DE-AC02-06NA25396.

210. **Development of wheat leaf rust, *Puccinia triticina*, ESTs from various spore stages**. Guus Bakkeren[1], Junhuan Xu[1], Rob Linning[1], Yehoshua Anikster[2], Brent McCallum[3], Travis Banks[3], Sarah Munro[4], Michael Mayo[4], Brian Wynhoven[4], Johar Ali[4], Richard Moore[4]. [1]Agriculture & Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC, V0H 1Z0, Canada; [2]Tel Aviv University, Israel; [3] Agriculture & Agri-Food Canada, Cereal Research Centre, Winnipeg, MB, R3T 2M9; [4] Michael Smith Genome Sciences Centre, Vancouver, BC, V5Z 4S6, Canada. bakkereng@agr.gc.ca

We are in the process of increasing genomic resources for the obligate wheat pathogen, the leaf rust fungus *P. triticina*. Approximately 17,000 EST reads covering a teliospore stage from senescent wheat and pycnio- and aeciospore stages from an alternate host infection on *Thalictrum speciosissimum* (meadow rue), were added to our previously established, 30,000 reads-large EST database (holding ESTs from urediniospore, appressorium and other wheat infection stages). These new spore stages contributed approximately 65%, 80% and 45% new unigenes to the existing database, respectively. From initial analyses it appears that aeciospores might express gene complements more similar to the urediniospore stages from wheat we sampled previously; this might illustrate the possible resemblance of function of these spores, i.e., infecting wheat. The pycniospores stage, which represents the sexual stage, displayed the largest pool of new genes. Other trends and comparisons will be presented.

211. **The secretome of *Coprinopsis cinerea.*** Andrzej Majcherczyk, Dorothea Fragner, Ravi Chandra Dwivedi, Mojtaba Zomorrodi, and Ursula Kües. Institute of Forest Botany, Georg-August-University, Göttingen, Germany

Basidiomycetes secrete many different proteins for degradation of complex substrates. In the complex fungal secretomes, only few proteins can be studied by direct detection of enzymatic activities and changes in substrate composition. To understand and to follow degradation processes, we use mass spectrometry for the analysis of tryptic digests of proteins with a Mascot database containing known protein sequences, annotated genomic sequences and ESTs from *Coprinopsis cinerea*, *Trametes versicolor* and *Pleurotus ostreatus*, respectively. Analysis of the *C. cinerea* secretome showed an increasing complexity with rising age of cultures. Several different glucosidases, oxidative enzymes (e.g., laccases, manganese-dependent peroxidases, glyoxal and glucose oxidases), lectin-like proteins and multiple proteolytic enzymes have been identified in liquid culture. Other enzymes effective in substrate degradation localize to the fungal cell wall and methods are developed to identify their individual nature. Part of this work is supported in frame of a Common Lower-Saxony-Israel Project (VW-Vorab). The Deutsche Bundesstiftung Umwelt financially supported our laboratory.

212. **Identification of pathogenicity factors among secondary metabolite-related genes in *Alternaria brassicicola.*** Kwang-Hyung Kim[1], Yangrae Cho[1], Mauricio La Rota[1], Robert Cramer[2], and Christopher Lawrence[1]. [1] Virginia Bioinformatics Institute and Department of Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. [2] Department of Molecular Genetics and Microbiology, Duke University Medical Center, Duke University, Durham, NC 27708-9902

Very little information is currently available concerning the pathogenic determinants produced by *Alternaria brassicicola*, the causal agent of black spot disease of Brassica plants. Production of secondary metabolites with host and non-host specific phytotoxicity has been proposed to play a role during the infection process of plants by fungi, especially in necrotrophic fungal pathogens. Gene clusters containing Polyketide Synthase (PKS) and Non-Ribosomal Peptide Synthetase (NPS) coding genes have been shown to be a major source of toxic secondary metabolites in fungi. To test the hypothesis that secondary metabolites produced by *A. brassicicola* contribute to pathogenicity, 8 NPS and 9 PKS genes were first identified by mining the *A. brassicicola* genome sequence database in our lab. Knockout (KO) mutants, constructed by gene disruption technology developed in our lab, were obtained for all 17 genes and evaluated for changes in fungal development and pathogenicity. Results of studies so far indicate some of the KO mutants of PKS and NPS genes showed interesting phenotypes and/or reduced virulence. Here we present functional characterizations of two interesting NPS genes, *AbNPS2* and *AbNPS4*. As a result of NCBI BLAST searches, AbNPS2 and AbNPS4 showed high similarity (E-value 0.0) to NPS4 and NPS12 of *Cochliobolus heterostrophus*, respectively. To further characterize the *abnps2* and *abnps4* transformants, we are conducting chromatographic and histomorphological analyses. Future studies include further characterization of other PKS and NPS genes, KO mutagenesis and plant pathogenicity analyses, and structural analyses (LC-MS and NMR) of the *in vitro*-produced metabolites identified thus far.

213. **Contributions of aneuploidy to fluconazole resistance in *Candida albicans.*** Anna Selmecki, Anja Forche, Judy Berman. University of Minnesota, Minneapolis, MN selme003@umn.edu

Genomic instability and aneuploidy are common in *Candida albicans*. Using comparative genome hybridization (CGH) microarrays, we found a strong correlation between aneuploidy and azole resistance[1]. Furthermore, we identified a segmental aneuploidy of chromosome 5L (Ch5L) DNA that was due to the formation of an isochromosome (a chromosome with two identical arms), in many fluconazole resistant (Flu^R) strains. Isochromosome 5L (i(5L)) formation is often accompanied by loss of heterozygosity (LOH) along Ch5L and while i(5L) is relatively stable under rich culture conditions, loss of i(5L) does occur and always correlates with a loss of Flu^R. Microarray analysis of i(5L) strains show that most genes on Ch5L exhibit increased expression and may contribute to Flu^R. Several well characterized genes involved in drug transport, *ERG11* and *TAC1*, are

present within the isochromosome and thus present in increased gene copies. We found that deletion of one or two extra copies of these genes in an i(5L) strain background has only partial effects on Flu^R, suggesting that increased copies of these genes alone are not responsible for the high level of resistance seen in the i(5L) strain. We are currently using telomere-mediated chromosome truncations of Ch5L to determine the degree to which different gene regions convey resistance. The effects of aneuploidy on gene copy number, gene expression level, LOH, and acquired azole resistance will be discussed. 1. Selmecki et al. (2006) Science 313: 367-370.

214. **Initial analyses of the transposable elements of *Rhizopus oryzae*.** Margi Butler[1], Manfred Grabherr[2], Tim Goodwin[1], Russell Poulter[1], Michael Koehrsen[2], Reinhard Engels[2], Sinead O'Leary[2], Chinnappa Kodira[2], Bruce Birren[2] and Li-Jun Ma[2]. [1] University of Otago, NZ. [2]Broad Institute of MIT and Harvard, Cambridge MA. margi.butler@otago.ac.nz

Rhizopus oryzae is the first Zygomycete fungus to be sequenced and differs from all previously sequenced fungal genomes in being highly repetitive. Repeat sequences account for approximately 35% of the genome. Such a heavily repetitive genome provides the unique opportunity to study the origin of the repeats and their impact on genome evolution. Analyses show that more than half of the identified repeats are derived from transposable elements. Representatives of all the mobile elements, including class I retrotransposons (LTR retrotransposons, non-LTR retrotransposons and DIRS-like elements) and class II DNA transposons (DDE transposons, Helitrons and Cryptons) are found in the genome assembly. Remarkably, many of the mobile elements are apparently functional. No evidence of RIP (repeat induced point mutation) was detected among copies of transposable elements. The presence of abundant, apparently functional, elements will provide a unique resource for analysing transposon and retrotransposon replication. We will describe the frequency, diversity and distribution of these elements in the genome and will discuss the their impact on the genome dynamics of organism. Funding for the *Rhizopus oryzae* genome sequencing was provided by NHGRI

215. **Identifying Divergent Genes in the Genus Neurospora and Related Species Using Comparative Genomic Hybridizations.** Luz B. Gilbert*[1], Takao Kasuga[1], Jeff Townsend [2], Louise Glass[1], John W. Taylor[1]. [1]Department of Plant and Microbial Biology, UC Berkeley, [2]Department of Ecology and Evolutionary Biology, Yale University, *lgilbert@berkeley.edu

Array based Comparative Genomic Hybrization or CGH is a useful tool to identify candidate genes that differ between species. Using the 70mer expression array designed for *Neurospora crassa* we compared seven species of *Neurospora*, as well as *Podospora anserina* and *Sordaria macrospora* in an effort to detect divergent genes. A second whole-genome tiling array was constructed by Nimblegen Systems for *Neurospora crassa* for a more comprehensive CGH analysis in both genic and intergenic regions of the genome. Two of the nine species used in the 70mer study were competitively hybridized to the Nimblegen tiling array. Genes found divergent using both arrays were classified according to functional category and a subset were selected for sequencing analysis.

216. **Whole transcriptome analysis of *Coprinus cinereus* meiotic development: a nosey look at mushroom sex.** Claire Burns[1]; Jason Stajich[2]; Jason Lieb[3]; Walt Lilly[4]; Allen Gathman[4]; Mimi Zolan[1]; Pat Pukkila[3]. [1]Dept. of Biology, Indiana University, Bloomington, IN, USA; [2]Dept. of Plant Biology, University of California, Berkeley, CA, USA; [3]Dept. of Biology, University of North Carolina, Chapel Hill, NC, USA; [4]SE Missouri State University, Cape Girardeau, MO, USA

Coprinus cinereus, a basidiomycete mushroom fungus ideally suited to meiotic studies due to its synchronous development, has a near-completed genome. EST information is available for *ca*. 2500 genes, and gene prediction algorithms indicate a total of *ca*. 12,500 genes. We have designed and fabricated a 13,230 element 70-mer oligonucleotide microarray using ArrayOligoSelector. Design criteria and array formulation will be presented. These arrays are to be made available to the wider *Coprinus* community. The tightly-regulated light and temperature dependent life cycle of the mushroom allows sampling of gill tissue in which cells are at a specific meiotic stage at a certain time. Meiosis occurs synchronously and takes *ca*. 12 hours. Our initial analyses using the new *Coprinus* arrays concern a broad 15-hour timecourse through the meiotic process, describing events prior to fusion of dikaryotic nuclei, nuclear fusion, condensation and alignment of meiotic chromosomes, first meiotic division, and completion of the second division. Analysis of resultant data using several algorithms, including ArrayLod and SAM, will be presented. This work is supported by NIH, and by the Indiana METACyt Initiative of Indiana University, which is funded in part through a major grant from the Lilly Endowment, Inc.

217. **Genome sequence of *Batrachochytrium dendroabatidis.*** Christina Cuomo[1], Manfred Grabherr[1], Lucia Alvarado[1], David DeCaprio[1], Chinnappa Kodira[1], James Galagan[1], Timothy James[2], Joyce Longcore[3], and Bruce Birren[1]. [1]Broad Institute of MIT and Harvard, Cambridge, MA [2]Uppsala University, Uppsala, SWE [3]University of Maine, Orono, ME

Batrachochytrium is a pathogen of amphibians implicated as a primary causative agent of amphibian declines. This recently emerging pathogen was identified in 1998 as the cause of amphibian deaths in Australian and Central America. More recently, *B. dendrobatidis* has been implicated in population declines of frog species in North America, South America, Europe and Africa.

We chose to sequence strain JEL423, isolated from a sick *Phylomedusa lemur* frog from Panama. We produced a 7.4X whole genome shotgun assembly, which contains 23.4Mb of sequence in 348 contigs, which are linked into 69 scaffolds. The sequence is highly repetitive

(~24%), but no significant similarity is found to previously characterized transposons. Progress in automated gene prediction will be described. This is the first representative of the Chytrid order to have its genome sequenced, and will provide the opportunity for comparisons across the fungal clade along with the sister animal clade.

218. **Withdrawn**

219. **Fungal Comparative Genomics – Progress under the Fungal Genome Initiative.** Christina Cuomo, Matthew Henn, Li-Jun Ma, Kurt Labutti, Sean Sykes, Manfred Grabherr, Evan Mauceli, Sante Gnerre, Matthew Crawford, Michael Koehrsen, Reinhard Engels, Lisa Larson, Phil Montgomery, Matthew Crawford, Jared White, Matthew Pearson, Clint Howarth, Qiandong Zeng, Chandri Yandava, Sinead O'Leary, Lucia Alvarado-Balderrama, Sarah Young, Dave DeCaprio, Chinnappa Kodira, David Jaffe, Chad Nusbaum, James Galagan, and Bruce Birren. The Broad Institute of MIT and Harvard, Cambridge MA, bwb@broad.mit.edu

The Fungal Genome Initiative was launched to generate genomic resources to promote research on organisms across the fungal kingdom. The FGI has been creating fungal genome sequences, annotations, and analytic tools to support studies of individual organisms and their evolutionary origins. To date the FGI has released genome sequence for 28 fungi, including fungi from all four major branches of the fungal tree. In addition, 35 approved fungi are currently in the sequencing queue. A primary focus of the FGI has been to promote comparative studies by sequencing clusters of related fungi and phenotypic variants of species to help interpret the biology of key models or pathogens. Recently initiated projects include major sequencing efforts around human pathogenic fungi, Candida albicans (5 species), dermatophytes (5 species), Coccidioides (14 new strains), and other dimorphic pathogens (9 strains of 4 species). Comparative sequencing projects around plant pathogenic Fusarium and Verticillium species are also underway. We will describe recent progress on sequencing as well as new tools and web sites to help make these comparative data most useful to the community.

220. **Sequencing and Comparative Analysis of *Candida* Genomes.** Christina Cuomo[1], Matthew Rasmussen[2], Manfred Grabherr[1], David DeCaprio[1], Lohith Kini[2], Chinnappa Kodira[1], Esther Rheinbay[2], Radek Szklarczyk[2], Geraldine Butler[3], Neil Gow[4], Joseph Heitman[5], Michael Lorenz[6], Andre Nantel[7], Manolis Kellis[1,2], and Bruce Birren[1]. [1]Broad Institute of MIT and Harvard, Cambridge MA [2]MIT Computer Science and Artificial Intelligence Laboratory, Cambridge, MA [3] University College Dublin, Dublin, Ireland [4] University of Aberdeen, Aberdeen, United Kingdom [5]Duke University Medical Center, Durham, NC [6]University of Texas Health Science Center, Houston, TX [7]National Research Council of Canada, Montreal, PQ, Canada

Analysis of an individual fungal genome such as *Candida albicans* can be greatly enhanced by comparison to genomes of related species. Comparative genomics highlights sequences conserved between species and can inform such basic questions as how genomes evolve, what genes make a species unique, and what genes and noncoding sequences are shared among species. As part of the Fungal Genome Initiative, we have sequenced five related *Candida* species: *C. albicans* (strain WO-1), *C. tropicalis*, *Lodderomyces elongisporus*, *C. guilliermondii*, and *C. lusitaniae*. We have released assemblies and preliminary gene sets from each genome. Additionally we have generated comparative genomic resources including whole genome alignments, protein families, species specific genes, and *Candida* specific genes. These resources are being utilized by a community based comparative analysis project which is currently underway. The growing set of *Candida* genome sequences allows comparisons across a range of evolutionary distances, enabling many different approaches to study the conservation of genes and regulatory elements as well as the evolution of these elements and genomic architecture within *Candida* species.

221. DelsGate, a robust and rapid gene deletion construction method for functional genomics. María D. García-Pedrajas[1†], Laura B. Kapa[1], Michael H. Perlin[2], and Scott E. Gold[1]. [1]Department of Plant Pathology, University of Georgia, Athens, GA [2], Department of Biology, University of Louisville, Louisville, KY [†]Estación Experimental del Zaidín, CSIC, Granada, Spain

With increasing availability of fungal genome sequences there is great demand for fast, simple methods to generate gene deletion constructs. We describe a method, named DelsGate, that combines PCR and Gateway cloning technology together with the I-SceI homing endonuclease to generate precise deletion constructs in a simple, universal and robust manner in just two days. These constructs may then be used to produce deletion mutants in the organism of interest. The initial version (DelsGate?) involved PCR of the entire ORF and 1 kb of 5' and 3' flanks, followed by self-ligation, inverse PCR and Gateway cloning to generate the final construct. A simplified improved version (DelsGate) was later devised consisting of PCR of only the 5' and 3' 1 kb gene flanks followed directly by *in vitro* Gateway cloning and final circular deletion construct generation by *in vivo* recombination in *E. coli*. For use in DeslGate we have produced vectors for transformation of Ascomycetes and the Basidiomycete fungus *Ustilago maydis*. We have tested the feasibility and reproducibility of the DelsGate approach by generating deletion constructs for 12

genes in *U. maydis*. Although not tested here DelsGate should be well suited for high-throughput approaches to gene deletion construction in fungal species.

222. **High throughput gene knockouts in Neurospora.** Patrick D. Collopy[1], Hildur V. Colot[1], Gyungsoon Park[2], Carol Ringelberg[1], Liubov Litvinkova[2], Susan Curilla[1], Lorena Altamirano[2], Norma Gorrochotegui-Escalante[1], John Jones[2], Katherine A. Borkovich[2], and Jay C. Dunlap[1]. [1]Department of Genetics, Dartmouth Medical School, Hanover, NH, USA [2]Department of Plant Pathology, University of California, Riverside, CA

Gene disruptions have been made in predicted open reading frames in the annotated *Neurospora crassa* genome using a high throughput procedure as part of an NIH-funded Program Project (P01)*. Knock-out (KO) cassettes were created by yeast recombinational cloning techniques and electroporation of *N. crassa* conidia can be performed in a 96-well format. In order to expedite molecular confirmation of KO mutant strains, we have developed a program (http://borkovichlims.ucr.edu/southern/) that allows automated identification of the appropriate restriction enzyme to use during Southern analysis. Additionally, we have developed and implemented a Laboratory Information Management System (LIMS; http://borkovichlims.ucr.edu/php/sLIMS.php) to keep track of the various steps of our gene KO processes. All plates and tubes used during the knockout procedure are labeled with barcodes and managed systematically. Completed *N. crassa* KO mutant strains are submitted to the Fungal Genetics Stock Center after targeted gene replacements are confirmed by Southern analysis. The list of submitted strains is available at the Neurospora genome project website (http://www.dartmouth.edu/~neurosporagenome/knockouts_completed.html). Use of these tools and our current progress in creating KO mutants will be presented. *Colot and Park et al., 2006. A high-throughput gene knockout procedure for Neurospora reveals functions for multiple transcription factors. PNAS 103:10352-10357

223. High-throughput mutation procedure for *Neurospora* genes. Lorena Altamirano[1], Luibov Litvinkova,[1], Gyungsoon Park[1], John Jones[1], Hildur V. Colot[2], Patrick D. Collopy[2], Norma Gorrochotegui- Escalante[2], Carol Ringelberg[2], Jay C. Dunlap[2] and Katherine Borkovich[1]. [1] Department of Plant Pathology, University of California, Riverside, CA [2] Department of Genetics, Dartmouth Medical School, Hanover, NH, USA

Gene deletions have been made in predicted open reading frames and genes in the annotated *Neurospora crassa* genome using a high throughput procedure as part of an NIH-funded Program Project (P01). Many steps of the process utilize a 96-well format, including construction of knock-out (KO) cassettes (using yeast recombinational cloning techniques), electroporation of *N. crassa* conidia and subsequent analysis of transformants. In order to expedite molecular confirmation of KO mutant strains, we have developed a program (http://borkovichlims.ucr.edu/southern/) that allows automated identification of the appropriate restriction enzyme to use during Southern analysis. Additionally, we have created and implemented a Laboratory Information Management System (LIMS; www.borkovichlims.ucr.edu/php/sLIMS.php) to track the various steps in the gene KO process. All plates and tubes used during the knockout procedure are labeled with barcodes and managed using the LIMS. Completed *N. crassa* KO mutant strains are submitted to the Fungal Genetics Stock Center after the gene replacements are confirmed by Southern analysis. The list of submitted strains is available at the *Neurospora* genome project website (http://www.dartmouth.edu/%7Eneurosporagenome/knockouts_completed.html). To date, we have constructed knockout mutants for1480 *Neurospora* genes and a total of 2233 mutant strains have been submitted to the FGSC. Another 960 genes are in various stages of the knockout procedure.

224. **The Comparative Fungal Genome Websites of the Broad Institute.** M. Crawford, M. Koehrsen, R. Engels, L. Larson, P. Montgomery, J. White, M. Pearson, C. Howarth, T. Ledlie, D. Park, D. DeCaprio, J. Galagan, B. Birren. Broad Institute, Genome Biology, Cambridge, MA

Efforts at the Broad's Fungal Genome Initiative focus on the sequencing of clusters of related organisms. To make these data as useful as possible we are developing a set of comparative group web sites to analyze multiple genomes. These sites feature redesigned statistics pages that highlight differences and similarities between different assemblies, genomes, and gene predictions. BLAST and feature searches can be performed simultaneously on the genomes of all the organisms in the group, with the results tabulated for meaningful comparison. The process for downloading has also been adjusted: sequences and other files generated for several organisms can be conveniently downloaded together. For group sites where the corresponding genes between organisms have been mapped, a set of views and tools facilate the analysis of related genes, including a Tree View and an Alignment view. The Argo browser now incorporates a comparative view that can assist in analyzing two or more genomes simultaneously. To make these tools as widely useful as possible we are integrating published genomes generated by others to give users a consistent analysis platform.

225. Tagging target genes of the MAT1-2-1 transcription factor in *Fusarium verticillioides (Gibberella fujikuroi* MP-A). Anita Keszthelyi[1], Apor Jeney[1], Zoltán[1], Odette Mendes[2], László Hornok[1] and Cees Waalwijk[2]. [1]Agricultural Biotechnology Center, Szent-Györgyi A. u. 4, H-2100 Gödöllõ, Hungary [2]Business Unit Biointeractions and Plant Health, Plant Research International BV, P.O. Box 16, 6700 AA Wageningen, The Netherlands cees.waalwijk@wur.nl

Mating type in filamentous ascomycetes is controlled by idiomorphic alleles, named MAT1-1 and MAT1-2, which contain one to three genes. Of these genes MAT1-1-1 and MAT1-2-1 encode putative transcription factors and are thus considered to be the major regulators of sexual communication and mating. Fungi with no known sexual stage may also have fully functional mating type genes therefore it was plausible to hypothesize that the MAT products may also regulate other types of genes not involved directly in the mating process. To identify putative target genes of these transcription factors in *Fusarium verticillioides*, DMAT1-2-1 knock out mutants were produced and transcript profiles of mutant and wild type were compared by means of differential cDNA hybridization. Clones, either up- or down- regulated in the DMAT1-2-1 mutant were sequenced and a total of 248 sequences were blasted against the NCBI database as well as the *G. zeae* and *G. moniliformis* genomes. Fifty-five % of the clones were down-regulated in the mutant, indicating that the MAT1-2-1 product positively affected these tagged sequences. On the other hand, 94 sequences (~45%) were found to be up-regulated in the mutant, suggesting that the MAT1-2-1 product also exerted a negative regulatory function on this set of genes. Sequences involved in protein synthesis and metabolism occurred more frequently among the clones, up-regulated in the mutant, whereas genes belonging to cell signalling and communication were especially frequently tagged among the sequences, down-regulated in the mutant.

226. Computational Predictions of Gene Structure in the Filamentous Fungus. *Aspergillus niger.* Christine Chee[1], Diego Martinez[1,2] and Mary Anne Nelson[1]. [1]Department of Biology, University of New Mexico, Albuquerque, NM 87131; cchee@unm.edu, manelson@unm.edu [2]Los Alamos National Laboratory/Joint Genome Institute, Los Alamos, NM 87018; admar@lanl.gov

The Department of Energy's Joint Genome Institute has recently sequenced the genome of the filamentous fungus *Aspergillus niger*. Like all eukaryotic organisms, *A. niger* has many genes with complex structure. To assist in the discovery of this large number of genes, the JGI utilizes a gene prediction pipeline to automatically predict the intron – exon structure of genes. All information is displayed on an interactive website, The JGI Genome Portal (http://genome.jgi-psf.org/index.html). Due to the complexities of gene structure, it is necessary to manually verify and correct about 25% of the predicted gene models; the most common errors are truncated 5' and/or 3' ends. The interactive Genome Portal allows for comparison of *A. niger* predicted proteins against the proteins of many other species simultaneously, as well as known partial cDNAs and mRNA information. If editing is required, the curator can adjust the gene model structure or create an entirely new gene model. The combination of sequence comparisons and editing ability allows the researcher to create the most accurate gene models currently possible.

227. An update on the *Neurospora crassa* community genome annotation project. Heather M. Hood[1], Matthew R. Henn[2], Dave DeCaprio[2], James E. Galagan[2], Alan Radford[3], Bruce W. Birren[2], Jay C. Dunlap[4], & Matthew S. Sachs[1]. [1]Oregon Health & Science University, Beaverton, OR 97006; [2]Broad Institute of MIT & Harvard, Cambridge, MA 02141; [3]University of Leeds, Leeds, UK; [4]Dartmouth Medical School, Hanover, NH 03755.

To produce a more accurate *Neurospora* protein-coding gene catalog, we need to include the wealth of information about gene structure and function that is contained within the scientific community. A web-based community annotation resource was developed and released in March 2006 to help accomplish this (http://www.broad.mit.edu/annotation/genome/neurospora/CAHome.html). Community annotators can use this site to add a variety of information to genome features, including both genetic and functional data. They can also correct gene models and add alternative transcripts. Immediately following submission, community annotations are appended to the gene detail page and are publicly available. Each submitted annotation is inspected for accuracy by curators; once curated, the annotation is scheduled to be incorporated into the official gene detail page as part of an updated genome release. A new release of *Neurospora* gene predictions (Version 3) included community input. As of December 2006, the community annotation project has 57 registered participants that have contributed over 500 annotations, including 130 citations. Over 1100 E-compendium entries were also incorporated as community annotations, further enriching the data set. Finally, a Textpresso database was implemented for *Neurospora* literature. This database contains over 2500 searchable full-text articles related to *Neurospora* and fungal biology. Taken together these new community resources extend the utility of the genome sequence data.

228. **Almost finished: the complete genome sequence of *Mycosphaerella graminicola*.** Gert H.J. Kema[1], Igor Grigoriev[2], Andrea Aerts[2], Asaf Salamov[2], Hank Tu[2], Harris Shapiro[2], Jim Bristow[2], Jane Grimwood[3], and Stephen B. Goodwin[4]. [1] Plant Research International B.V., P.O. Box 16, 6700 AA Wageningen, The Netherlands. [2] Department of Energy, Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA, 94598, USA. [3] Stanford Human Genome Center, 975 California Avenue, Palo Alto, CA 94304, USA. [4] USDA-ARS, Purdue University, 915 West State Street, West Lafayette, Indiana, 47907-2054, USA.

Mycosphaerella graminicola is a haploid ascomycete causing septoria tritici blotch of wheat. An 8.9x shotgun sequence of bread wheat strain IPO323 was generated through the Community Sequencing Program of the U.S. Department of Energy's Joint Genome Institute (JGI), and was finished at the Stanford Human Genome Center. The finished genome of M. graminicola contains 39.6 Mb. All available ESTs (37,747) were placed on the finished assembly to assess completeness. In addition, 1793 Diversity Array Technology markers were sequenced at JGI and aligned with the physical map. The genetic and physical maps showed near-perfect colinearity, demonstrating the high quality of both the genetic linkage map and the genome assembly.

Fifteen scaffolds are complete and represent entire chromosomes with sizes from 548 kb to 6 Mb. In addition, six scaffolds (ranging from 21 kb to 2.4 Mb) contain a single telomere and could connect in any combination. Two additional scaffolds do not contain telomeres and only 17 gaps remain to be closed. Our sequence data suggest that isolate IPO323 contains 18-20 chromosomes, which represents the highest number of chromosomes reported among ascomycetes. This conclusion is corroborated by high-density genetic linkage maps and detailed pulsed-field gel analyses. The complete mitochondrial sequence is a circular genome of 43,947 bp. The machine annotation predicted 13,413 genes, of which 1126 have been annotated manually (http://www.jgi.doe.gov/Mgraminicola). Gene annotations generally indicate that *M. graminicola* has fewer genes per family than other plant-pathogenic filamentous fungi, which may reflect its initially extracellular biotrophic and subsequently necrotrophic lifestyle. This genome represents the first nearly finished genome of any filamentous plant pathogen and is of great importance for comparative genomics involving other Dothideales, such as the destructive banana Black Sigatoka pathogen *Mycosphaerella fijiensis* that currently is sequenced at the ~7.1x level.

229. **Improvements in *Aspergillus fumigatus* annotation**. Paolo Amedeo, Natalie Fedorova, Rama Maiti, Vinita Joardar, Jonathan Crabtree, Samuel Angiuoli, William Nierman, Owen White, Jennifer Russo Wortman. The Institute for Genomic Research 9712 Medical Center Dr., Rockville, MD 20850

Aspergillus fumigatus was among the first fungal genomes to be fully sequenced and annotated. At the time, few characterized genes in closely-related organisms were available in the public databases, which had a negative effect on the quality of annotation. Since then annotation tools have improved and several other *Aspergilli* have been fully sequenced and annotated. We have revised the annotation of *Aspergillus fumigatus* leveraging comparative analysis techniques and the newly-available data from related organisms. Here we describe these processes and discuss improvements made to the annotation.

230. Withdrawn

231. **Mitochondrial Genomics in the Genera *Pythium* and *Phytophthora*.** Frank N. Martin[1] and Paul Richardson[2]. [1]USDA-ARS, Salinas, CA, [2] Joint Genomics Institute, Walnut Creek, CA.

Mitochondrial genomics can be useful for investigating the processes contributing to evolutionary divergence at a mtDNA sequence level and hence, provide insight to the phylogenetic relationships of the organisms under study. While the mitochondrial genomes of the related genera *Pythium* and *Phytophthora* encode a similar suite of genes, they differ from each other by the presence of a large inverted repeat (IR) that is found in *Pythium* (it can represent approximately 80% of the genome size). In an effort to gain a better understanding of the evolutionary forces responsible for sequence divergence in genomes with and without an IR, as well as to clarify the phylogenetic relationships within the individual genera, the mitochondrial genomes of 15 *Pythium* and 10 *Phytophthora* species were sequenced. Comparative genomics among species within a genus indicated that certain regions of the genome were more polymorphic than others. In *Pythium*, the most polymorphic region was the small unique region and adjacent IR sequences. In *Phytophthora* genomic inversions were observed with many of the rearrangements corresponding to phylogenetic groupings. Two closely related species (*P. ramorum* and *P. hibernalis*) also were found to have small IRs (1.1-1.5 kb in size). While the IR in *Pythium* appeared to stabilize the genome from rearrangements, the data suggests that the rate of evolutionary divergence was more dependent on the specific gene rather than its location within the IR.

232. Withdrawn

233. **Transcriptome of conidium and ascospore development in *Fusarium graminearum*.** Kye-Yong Seong[1], Matias Pasquali[1], Jin-Rong Xu[2], and H. Corby Kistler[1,3]. [1]University of Minnesota, St. Paul, MN, USA; [2]Purdue University, West Lafayette, IN, USA; [3]USDA ARS Cereal Disease Laboratory, St.Paul, MN, USA.

To understand the infection cycle of the head blight pathogen *F. graminearum*, gene expression profiles were monitored during both ageing and germination for conidia and ascospores. Ascospores and conidia were treated in a similar manner, either aged under desiccating conditions or suspended in liquid germination medium. RNA was extracted from cultures and used to query the 18K feature *F. graminearum* Affymetrix GeneChip. Overall, a slightly greater number of probe sets corresponding to genes were detected in ascospores (9,207) than in conidia (8,815; detection p value <0.001) but the majority of probe sets (8,068) were shared between conidia and ascospores. While a similar number of genes were detected at most stages of development, the biggest difference among spore types was upon desiccation where the number of probe sets detected in ascospores (6,801) was more than twice the number detected in conidia (2,916). These results indicate that ascospores remain more metabolically active than conidia upon ageing. Peroxisomal proteins and genes involved in lipid beta-oxidation are strongly up-regulated both in fresh conidia and in ascospores. After suspending conidia or ascospores in liquid germination medium, numerous genes involved in transcription, RNA splicing, protein synthesis, and amino acid and nucleotide metabolism were highly induced. Up-regulation of proteasome components and secretory proteins were observed as spores established polarized growth after 8h of incubation. Comparing gene expression in spores with expression in hyphae under a variety of environment regimes indicates that a total of

328 probe sets were specific for ascospores and another 150 were specific for conidia. Spore-specific gene expression may be used to develop hypotheses concerning spore maturation, dormancy and initiation of germination. This work is supported by the National Research Initiative of the USDA CSREES, award 2004-35604-14327.

234. **Restriction-site Associated DNA (RAD) mapping can be used to rapidly map mutations using Neurospora microarrays.** Zachary A. Lewis, Anthony Shiver, Michael Miller, Eric A. Johnson, and Eric U. Selker. University of Oregon, Eugene, Oregon

Many important insights into fundamental biological processes have come from the study of filamentous fungi. The wealth of sequence information currently available for this group has facilitated evolutionary analysis, genome level expression analysis, and reverse genetic studies. However, the ability to carry out forward genetic screens and selections continues to drive discovery in the fungi. Whereas isolation of mutants is relatively easy, mapping and identification of the mutant genes is often the "rate limiting" step in genetic studies. We have performed a selection for genes that are defective in DNA methylation (dim). In order to identify the mutant genes, we have adapted microarray-based RAD mapping for use with Neurospora oligo arrays. We demonstrate that this technique can be used to detect genome wide restriction site polymorphisms from two polymorphic Neurospora crassa strains, Mauriceville and Oak Ridge. Moreover, we demonstrate that this technique can be used to rapidly map a mutation. RAD mapping can be used to identify restriction site polymorphisms in any two polymorphic strains without a priori knowledge of the polymorphisms. Therefore, in addition to forward genetics, we propose that RAD mapping will useful for analysis of phenotypic variation in natural populations (e.g. QTL analysis).

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235. **Targeted Introduction of Point Mutations in *Epichloë festucae*.** Kurtis Knapp, Richard Johnson, Christine Voisey, and Gregory Bryan. AgResearch Ltd.

AgResearch has a number of proprietary endophytes that increase plant yield and provide resistance to abiotic stress. However, these strains can not currently be commercialized because they produce mammalian toxic alkaloids. We will eliminate production of these alkaloids using a point mutagenesis technique called Rapid Trait Development System (RTDS). In RTDS conversions, one transforms an oligonucleotide that hybridizes with the gene of interest but contains a single base mismatch. The cell then alters the mismatched base in the genome. This technique is attractive to agricultural biotechnology because it avoids the untoward changes of random mutagenesis and the regulatory issues of transgenics. We will present progress toward implementation of RTDS to fungal endophytes of grasses. Initial work will be on mutations with a selectable phenotype in the *Epichloë festucae* strain FL1.

236. **New screening approaches for fungal strain development.** Peter J. Punt[1], Xavier O. Weenink[2], Marc van der Maarel[1], Jan Jore[1], Arthur Ram[2], Cees van den Hondel[2]. [1]TNO Quality of Life, Zeist, the Netherlands,[2]Leiden University, Leiden, the Netherlands

Since the development of recombinant DNA technologies for yeast and filamentous fungi, a considerable part of the strain development programs was diverted to the use of molecular genetic tools. Whereas these approaches have exerted considerable success, recent developments in our laboratory have shown that new developments in classical biological screening approaches, or a combination of both, can still be very useful. A first purely classical approach is based on the discovery of a so-called suicide (SUI) substrate, which we have successfully used for the selection of protease deficient fungal host strains. These protease deficient strains show an increased resistance to the SUI substrate allowing their selection. The advantage of this non-GMO approach is that it can be applied to new and already established production strains. A combination of a molecular and classical approach is based on the use of the so-called glucoamylase carrier approach. Combining this approach with fungal strains unable to use starch as a carbon source allowed us to select for hyper secretive fungal strains generated by classical mutagenesis (Weenink et al., 2006). Moreover, the same approach also allows for selection of the highest producers in a collection of primary transformant strains expressing a glucoamylase-fusion gene.

237. **Oxidative protein folding in *Aspergillus niger*.** Anna Harvey[1], Andrew Plumridge[1], Mick Ward[2], Huaming Wang[2] & David B Archer[1]. [1]School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD [2]Genencor Intl., 925 Page Mill Road. Palo Alto, CA 94304, USA plxarh@nottingham.ac.uk

The formation of protein disulphide bonds is one of several processes that occur within the endoplasmic reticulum (ER) of eukaryotes, which can be vital for the correct folding of secreted proteins. The pathway for the formation of protein disulphide

bonds has been characterised in the yeast model organism, *Saccharomyces cerevisiae*. In *S. cerevisiae*, two proteins, Ero1p and Pdi1p are essential for the correct formation of protein disulphide bonds, and their expression is regulated by ER stress through the unfolded protein response. In the filamentous fungus, *Aspergillus niger*, the presence of a Pdi1p homologue, PDIA, has already been demonstrated. We have identified a homologue of the Ero1p protein and we show that its expression is transcriptionally regulated by ER stress. The functions of *eroA* and the product EROA are being further characterised by investigating the effects of deletion and over- expression.

238. ***Chrysonilia sitophila*: a Case Study for Ascomycete Biodegradation and Bioremediation Potential.** Carvalho, MB[1,a], Martins, I[1,a], Garcia, H[2], Spencer-Phillips, P[3], Gil, A[2], San Romão, MV [1,4] and Silva Pereira, C [1,*] [1]ITQB-UNL/IBET, Instituto de Tecnologia Química e Biológica - Universidade Nova de Lisboa / Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal. [2]Universidade de Aveiro, Departamento de Química,Aveiro, Portugal [3]University of the West of England, Bristol, UK [4]EVN, Estação Vitivinícola Nacional, Dois Portos, Portugal. * Corresponding author, spereira@itqb.unl.pt [a] Equal contributing authors

Persistent organic pollutants, such as pentachlorophenol (PCP), are globally recognized as a major environmental concern. PCP constitutes an acute risk to health considered, by the WHO (2004), as one of the most hazardous substances. PCP use is nowadays wide restricted but is still frequently detected in all environments. Whole fungal cell biocatalysis represents an elegant way for elimination of extreme toxic products. However, fungi exploitation for biodegradation is often sub-optimal and rarely reliable. One of the underlying reasons for the fragility of many approaches is the lack of scientific knowledge concerning the diversity, activity and dynamics of the microbial communities responsible for the biodegradation processes. There is a close relation between fungi biodegradation and bioremediation abilities because the aromatic matrix of lignin resembles the basic molecular skeleton of PCPs. During cork stopper manufacturing process, cork slabs are initially colonised by *Chrysonilia sitophila* and latter by other fungal species, such as *Penicillium glabrum* and *Trichoderma longibrachiatum.* These fungal species are able to perforate the complete thickness of the cork cell wall (lignin 20%, suberin 40%), suggesting their active biodegradation ability and were able to degrade a less, relative to PCP, chlorinated chlorophenol. Fungi biodegradation ability was screened following cork decay by spectroscopy (FITR). *C. sitophila* degrades lignin and suberin, tolerates concentrations of PCP above 20 mg/L and can apparently grow solely in the presence of PCP. Growth media supplementation with lignin/suberin enriched composite apparently reduces PCP toxicity. PCP degradation pathway by *C. sitophila* is being analysed following PCP degradation intermediates formed and the fungi proteome during growth on different substrates (presence and absence of PCP).

239. **Bottlenecks in Bacterial Transglutaminase Production in *Trichoderma reesei*.** Marja Paloheimo[1], Susanna Mäkinen[1], Kim Langfelder[2] and Jari Vehmaanperä[1]. [1]Roal Oy, Rajamäki, Finland. [2]AB Enzymes GmbH, Darmstadt, Germany.

Transglutaminases catalyse cross-linking in proteins via an acyl transfer reaction between peptide-bound glutamine and lysine. TGases are widespread in mammals and they have also been found in microorganisms, plants, invertebrates, fish and birds. The cross-linking activity of TGases is exploited in several food applications, e.g. in improving the texture of processed meat and fish products. There is a growing interest to use TGases in technical applications, such as use in the textile industry. TGases could be used in modification of silk and wool fibres. For technical applications a cheap source of TGase must be available. For this reason a preliminary study was performed to determine whether *Trichoderma reesei* is a suitable host for production of a bacterial transglutaminase. The *S. mobaraensis* tg gene was used as a test gene. The TGase is activated after secretion by two-step cleavage of the pro-peptide from the precursor. Constructs encoding the full-length and mature and the corresponding inactive TGase forms were expressed in *T. reesei*. In addition the pro-sequence was expressed separately in strains already expressing the mature form of TGase. The TGase encoding sequences were expressed in *T. reesei* as 3´-fusions to a sequence encoding a carrier polypeptide. The results obtained will be shown and discussed.

240. **Targeting cellular stress response systems with natural compounds to enhance antifungal activity.** Jong Kim[1], John Beck[1], Shen-Chieh Chou[2], Noreen Mahoney[1], Kathleen Chan[1], Russell Molyneux[1] and Bruce Campbell[1*]. [1] Plant Mycotoxin Research, USDA, Albany, CA 94710 USA. bcc@pw.usda.gov. [2] School of Pharmacy, University of Colorado Health Science Center, Denver, CO 80262.

A series of natural phenolic compounds (i.e., benzoic/cinnamic acid derivatives, etc.) were applied to various gene deletion mutants of *Saccharomyces cerevisiae* in order to identify gene targets for fungal control. Four groups of molecular targets were identified, as follows: transcription regulators of pH response or glutathione transferase; vacuolar H(+)- ATPase; mitogen-activated protein kinase kinase (MAPKK); and antioxidative enzymes. Treatments with 2,3- dihydroxybenzoic acid disrupted fungal glutathione homeostasis. Several compounds targeted mitochondrial superoxide dismutase (Mn-SOD). Functional complementation analysis using *Aspergillus flavus sodA*, encoding Mn-SOD verified increasing oxidative stress improved antifungal activity of commercial fungicides. Activity of strobilurin was greatly elevated on *A. fumigatus* and *A. nidulans* by co-application with berberine or veratraldehyde. These compounds also prevented *A. fumigatus* MAPK mutants from escaping toxicity of fludioxonil. Esterification of dihydroferulic acid (DFA) with methyl, ethyl, or propyl side chains greatly enhanced antifungal acitivity. We conclude that targeting fungal antioxidative stress systems with phenolic agents improves fungal control.

241. Withdrawn

242. **Ceramidase CerA of *Aspergillu oryzae* promotes degradation of biodegradable plastic containing urethane bonds.** Shinsaku Ohtaki[1], Hiroshi Maeda[2], Toru Takahashi[1], Youhei Yamagata[1], Katsuya Gomi[1], Fumihiko Hasegawa[2], and Keietsu Abe[1, 2*]. [1]Grad. Sch. of Agricul. Science, [2]New Industry Creation Hatchery Center, Tohoku University, Japan.

When the industrial fungus *Aspergillus oryzae* is grown in a medium containing a biodegradable plastic polybutylene succinate-co-adipate (PBSA) as a sole carbon source, the fungal cells secret several proteins including cutinase CutL1 as a PBSA degrading enzyme and several biosurfactant proteins that bind to the surface of PBSA and recruit CutL1 to promote PBSA degradation. From the culture broth, we found and purified another novel protein having a molecular mass of 100 kDa. Because the purified protein is predicted to be a neutral ceramidase by using *A. oryzae* genome information, the gene encoding the 100 kDa protein is designated *cerA*. We cloned the *cerA* gene from *A. oryzae* and constructed a recombinant *A. oryzae* strain highly expressing *cerA*. Purified recombinant CerA hydrolyzed C12-NBD-ceramide. Although CerA scarcely cleaved ester bonds between succinate and 1,4-butanediol in PBSA, CerA promoted degradation of PBSA in combination with CutL1. Since PBSA contains urethane bonds in every 200-300 butylene-succinate ester units and the urethane bonds are similar to the amide bonds in ceramides, CerA seems to degrade PBSA endogenously at the urethane bonds and to be applicable degradation of ester polymers containing urethane bonds.

243. **Characterization of novel thermostable fungal cellobiohydrolases.** Terhi Puranen[1], Sanni Voutilainen[2], Matti Siika-aho[2], Jarno Kallio[1], Satu Hooman[2], Liisa Viikari[2], Anu Koivula[2], and Jari Vehmaanperä[1]. [1]Roal Oy, Rajamäki, Finland, [2]VTT Technical Research Centre of Finland, Espoo, Finland

Cellulose degradation requires sequential or simultaneous synergic action of three types of hydrolytic enzymes: cellobiohydrolases, endoglucanases and beta-glucosidases. Various filamentous fungi produce these enzymes in order to hydrolyze insoluble cellulose into glucose. Cellulases are currently extensively studied in enzyme industry for cellulosic biomass conversion to ethanol. Here, molecular cloning of three different cellobiohydrolase genes from thermophilic ascomycetes is presented together with their heterologous expression in *Trichoderma reesei*. The recombinant cellobiohydrolases that belong to the glycosyl hydrolase (GH) family 7 were purified and characterized in terms of pH optimum, thermal stability and kinetic parameters. Thermostable cellulases such as described here have been proposed to improve the overall process economy of the biomass conversion with favourable impact on enzyme need, hydrolysis performance and flexibility of the process.

244. **Identification and characterisation of the *Trichoderma reesei* homologue of *Aspergillus nidulans creB*.** Jai Denton, and Joan Kelly. University of Adelaide, Adelaide, Australia.

Trichoderma reesei is an industrially significant organism, particularly in the cellulase production industry. The industrial hyper cellulase producing strain RUT-C30 was found to contain a mutation within the coding region of the *T. reesei* homologue of the carbon catabolite repressor, CreA. The carbon catabolite repression gene (CCR) *creB* has been previously implicated in the regulation of genes encoding enzymes involved in cellulose utilisation. In *Aspergillus nidulans*, disruption of the *creB* gene results in deregulation of cellulase encoding genes normally subject to strong CCR, without the severe reduction in mycelial growth that is present in *creA* disruptions. The *T. reesei* homologue of *creB* is an ideal candidate for disruption for the development of a commercially exploitable strain, since no extreme effects on morphology are predicted. A homologue, labelled *cre2*, was identified within the *T. reesei* genome. In order to test for complementation of an *A. nidulans creB* mutant strain, the *T. reesei cre2* gene was amplified and used to transform an *A. nidulans* strain containing a *creB* null mutation. The *T. reesei cre2* gene complemented the effect of the *creB* mutation on utilisation of quinate and proline, and sensitivity to allyl alcohol in the presence of glucose. A disruption of *cre2* was generated through the introduction of a selectable marker within the coding region. The disrupted strain was found to deregulate genes normally subject to CCR.

245. Withdrawn

246. **Optimization of cellulase expression in *Trichoderma reesei* for the production of cellulose ethanol.** Rebecca D. Taylor[1] (taylorr@agr.gc.ca), Anne Johnston[1], Daneille Schneiderman[1], Jiro Hattori[1], Nick Tinker[1], Linda Harris[1]. [1] Agriculture and Agri-food Canada, Eastern Cereal and Oilseeds Research Center, Ottawa, Ontario Canada K1A 0C6. Alex Sobko[2], Wei Ling Tan[2], Jason Edwards[2], Chris Hindle[2], Theresa White[2]. [2] IOGEN Corporation, 310 Hunt Club Road East, Ottawa, Ontario, Canada K1V 1C1.

The fungus *Trichoderma reesei* is used by IOGEN Corporation to produce cellulase enzymes for the production of cellulose ethanol from farm silage (wheat straw, corn stalks) and other biomass. The goal of this project was to analyze gene expression changes in *T. reesei* upon overexpression of cellulase proteins. To this end, we acquired 9298 high quality expressed sequence tags (ESTs) from 10 cDNA libraries constructed from fungal cultures grown under varied conditions. We also directly PCR

amplified additional genes of interest not present in our EST population. In total, we have 2979 unique sequences including 899 contig and 2080 singleton sequences representing approximately one third of predicted *T. reesei* genes. cDNA microarrays were constructed using these unique sequences. Biological samples for array hybridizations were harvested from 14L *T. reesei* batch fermentation cultures in the presence of soluble cellulase inducer. The results from these experiments will be discussed.

247. **Engineering of a novel protein secretion pathway in *Aspergillus niger*.** Robbert Damveld[1], Miranda Hartog[1], Peter ten Haaft[1], Inge Minneboo[1], Cees Sagt[1], Han de Winde[1], Thibaut Wenzel[1]. [1]DSM Food Specialties, P.O. Box 1, 2600 MA Delft, The Netherlands.

Production of enzymes on an industrial scale is often limited to extracellularly enzymes. When enzymes are produced intracellular, the downstream processing is costly and process robustness is not guaranteed. We have developed a technology which enables the secretion of enzymes which are normally localized in the cell. The folding conditions of intracellular proteins differ from those of secreted proteins. In the cytosol proteins fold under relative reducing conditions compared to the oxidizing environment in the ER. Moreover, many secreted proteins undergo extensive N- glycosylation and disulphide bridge formation. The set of folding enzymes and chaperones in the secretory pathway and in the cytosol are different as well. Therefore the simple solution of forcing intracellular proteins through the secretory pathway does in many cases fail to result in biologically active secreted enzymes. We have developed a method to secrete intracellular enzymes in an active form. This technology allows the intracellular enzymes to be folded in their native environment in the cytosol using cytosolic folding enzymes and chaperones. After folding the intracellular proteins are translocated to a modified cell compartment. After translocation in this modified cell compartment the content is released into the medium by a specific process. Using controlled fermentations we have established that the physiology of cells containing this novel secretory system is not dramatically different form non- modified cells. We will show the recently obtained proof of principle of this concept with the Green Fluorescent Protein (GFP). In addition we have demonstrated the concept for intracellular enzymes, which are secreted in an active form using this novel approach. Moreover we also have preliminary evidence that this technology can be used to secrete metabolites, which are normally localized intracellular. This is the first report that describes the introduction of a completely novel secretory pathway in eukaryotic cells, which allows the production of intracellular enzymes in a secreted active form enabling industrial application on an economically feasible scale.

248. **A transiently disrupted non-homologous end joining pathway in *Aspergillus nidulans* allows simple and efficient gene targeting.** Jakob Blæsbjerg Nielsen, Michael Lynge Nielsen, and Uffe Hasbro Mortensen. Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark, Denmark jbn@biocentrum.dtu.dk

Gene targeting was developed more than a decade ago for high-throughput gene deletions in the unicellular *Saccharomyces cerevisiae* owing to the fact that DNA double strand breaks are effectively repaired by the homologous recombination (HR) pathway. In multicellular eukaryotes, from filamentous fungi to man, DNA double strand breaks are preferentially repaired by the non-homologous end joining (NHEJ) repair pathway. When performing gene targeting in such multicellular organisms, the influence of NHEJ compromises the efficiency by causing random integration of the gene targeting DNA. Elimination of NHEJ by deleting one of the main genes in the pathway, e.g. *ku70*, *ku80* or *lig4* has been done in several fungal species including *Aspergillus fumigatus*, *A. nidulans*, *Sordaria macrospora* and *Neurospora crassa*. In such strains, the gene targeting efficiency is often as high as 100%. The phenotype of NHEJ strains includes altered colony morphology and an increase in sensitivity to various genotoxins. This raises the concern that phenotype of NHEJ deficient strains may produce synthetic effects with other mutations introduced in this background. This may hamper direct interpretation of the phenotype of this new mutation. In line with this, authors often recommend as a safety measure to reconstitute the NHEJ pathway, e.g. by retransforming with the functional gene or sexual crossing with wild-type strains, before analyzing the effects of novel mutations. In both cases, strain reconstruction constitutes a bottle neck in large scale gene-targeting experiments. To bypass these problems, we have developed an effective alternative in *A. nidulans*. The system employs a strain with a transiently disrupted *ku70* homolog as the starting point for efficient gene targeting. The *ku70* mutation in this strain can via a simple selection scheme be reverted to wild type after the desired genetic manipulation(s) have been carried out. The system can easily be adapted to other filamentous fungi.

249. **Applied genome-scale modelling of *Aspergillus niger*.** Mikael Rørdam Andersen, Michael Lynge Nielsen, Jens Nielsen. Center for Microbial Biotechnology, Build. 223, Technical University of Denmark, DK-2800 Lyngby, Denmark, jn@biocentrum.dtu.dk

The filamentous fungus *Aspergillus niger* has through the years fascinated academic and industrial researchers alike due to its innate ability to produce a large number of enzymes and high concentrations of acids. However, the regulatory mechanisms governing these processes are not thoroughly understood. We are combining genome-scale stoichiometric modeling, transcriptomics and novel tools for graphical analysis to elucidate these mechanisms. A genome-scale model was constructed based on literature of *A. niger* and related aspergilli. Completion of pathways was performed using pathway databases. Genomic

information was added based on the sequencing and annotation provided by DSM Food Specialities (Pel *et al*, manuscript accepted). The model comprises 1230 biochemically unique reactions (isoenzymes not included). 850 are supported by literature and 873 are backed by the genomic evidence of 1024 open reading frames. The remaining reactions are transport reactions and other reactions added for connectivity. Using a digital pathway map of the model, the computed metabolic fluxes were analysed for a large number of substrates. The results are in accordance with the available literature. Using a fast PCR-based cloning-free approach (Nielsen *et al*, 2006) adapted for *A. niger*, deletion mutants of carbon metabolism regulators of *A. niger* have been constructed. These are to be analysed using steady-state cultures and DNA arrays for *A. niger*.

250. Withdrawn

251. ***Coprinopsis cinerea* as a host for recombinant laccase production.** Sreedhar Kilaru[1,2], Martin Rühl[1], Patrik J. Hoegger[1], Aletta Grimrath[1], Karin Lange[1], Mojtaba Zomorrodi[1], Andzrej Majcherczyk[1], and Ursula Kües[1]. [1]Institute of Forest Botany, Georg-August-University, Göttingen, Germany [2] School of Biological Sciences, University of Bristol, Bristol, UK

Laccases (EC 1.10.3.2) from basidiomycetes are used for various industrial applications, e.g. in paper pulp bleaching, bioremediation, textile dye decolourization, and wood composite production. For overproduction of laccases, we developed a recombinant expression system using the basidiomycete *Coprinopsis cinerea* as a host. Various homologous and heterologous laccase genes have by now been expressed in *C. cinerea* under control of the *Agaricus bisporus gpdII* promoter. Yields of enzyme activities differ from gene to gene. Alteration of growth conditions (media, temperature, aeration) improved yields with 20fold increases in the best cases. At our current best cultivation conditions and our best gene, laccase activities of up to 30 U/ml are obtained. Individual enzymes are characterized in their properties and found to differ from each other, making them attractive for different biotechnological applications. Our laboratory is financially supported by the Deutsche Bundesstiftung Umwelt (DBU).

252. **Multivalent Influenza antigens produced in *N. crassa*, using a novel application of heterokaryon fusion technology**. Edward B. Cambareri, Yuliya Brudnaya and W. Dorsey Stuart Neugenesis Corporation, 849 Mitten Rd, Burlingame, CA 94010

Neugenesis Corporation has adapted patented technologies, developed to produce multimeric subunit proteins such as human IgGs, for use in the production of multivalent vaccines. Individual glycoprotein antigens or antigen variants can be expressed in different strains of the fungus, and then fused to create a heterokaryon strain that can produce either a mixture of secreted soluble antigens or high molecular mass particles that display the antigens. New production strains can be generated in as little as six weeks, and different antigens can be mixed and matched using the inherent combinatorial properties of fungal strain fusion. Commercial levels of tailored antigen mixtures can be rapidly isolated from simple, defined growth medium. Data will be presented demonstrating production from heterokaryons of a pandemic influenza vaccine candidate; a virus-like particle (VLP) displaying active H5 Hemagglutinin and N1 Neuraminidase glycoproteins.

253. **Isolation of isoosmotic up-regulated genes in *Aspergillus oryzae* and use of its promoters for protein expression system**. Ken Oda[1], Kazutoshi Sakamoto[2], Toshihide Arima[3], Yuka Okita[2], Dararat Kakizono[2], Osamu Yamada[2], Shinichi Ohashi[1], Osamu Akita[5], Kazuhiro Iwashita[2,4]. Kanazawa Institute of Technology[1],3-1 Yatsukaho, Hakusan, Ishikawa, Japan tel:D+81(76)274-7500, fax+81(76)274-7511, e-mail:Fodaken@neptune.kanazawa-it.ac.jp National Research Institute of Brewing[2], Prefecture Univ. of Hiroshima[3], Hiroshima Univ.[4], Jissen Women's Univ.[5], Japan

The mechanism of response to osmotic pressure exists in eukaryotes. In *A. orzyae*, response to osmotic pressure is significant factor for environment recognition in koji-making (solid-state culture), and in fact it was suggested that osmotic pressure regulation component, such as AtfA, or HogA, is important. We analyzed the genes which respond to isoosmotic pressure (0.8M NaCl and 1.2M sorbitol) by microarray analysis. In isoosmotic condition 96 genes in 3000 spots array were 2-fold up- regulated from 30 to 90 min after induction. Some genes that are needed to respond to osmotic stress, such as *atfA*, , *gpd*, , and *srk1*, were isolated. 19 genes were over 5-fold up-regulated, and named as Isoosmotic Up-regulated Genes (IUG). 5'-upstream region of 10 genes in IUG were cloned and applied to GUS reporter assay to evaluate induction ability in isoosmotic condition. The promoters of two genes (IUG2 and IUG9) were strictly regulated. To construct protein expression system the promoter region of high expression vector pNGA142 was substituted with IUG2 and IUG9 promoters. EGFP as a model of heterologous protein was expressed after 2hr only in induction conditions, suggesting that response speed of this system is fast and control of expression is strict. We constructed new protein expression system which protein expression was induced by osmotic pressure and their level of expression are controlled by NaCl concentration.

254. **Chemostat cultivation of Aspergillus niger for C-source mediated induction of the unfolded protein response and transcriptome analysis.** Theo Goosen[1,2], Thomas Jørgensen[1,3], Jens Iversen[1,3], Arthur Ram[1] and Kees van den Hondel[1]. [1]Leiden University, Institute of Biology Leiden, Fungal Genetics Research Group, Wassenaarseweg 64, 2333 AL Leiden, The

Netherlands, [2]BioCentre, HAN University, Laan van Scheut 2, 6525 EM Nijmegen, The Netherlands, [3]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, 5230 Odense, Denmark.

Folding of nascent proteins in the endoplasmic reticulum (ER) has been identified as a major bottleneck in production of heterologous proteins. Accumulation of unfolded proteins in the ER elicits a stress response called the unfolded protein response (UPR). We have used C-limited chemostat cultures of Aspergillus niger to investigate UPR resulting from expression of a gene encoding a mammalian antibody fragment (scFv). Chemostat cultivation provides well-defined growth conditions and reduces biological sample variation among replicates, which make it an excellent method for both physiological and molecular studies, like transcriptomics. Two strains were cultivated for comparison. Strain ABGT1026 expresses lama scFv under transcriptional control of the glaA promoter from a single targeted gene. Strain AB94-85 is an isogenic control strain lacking the scFv gene-fusion. Both strains were cultured in chemostat cultures, at a dilution rate of 0.16 h-1, initially with xylose-limitation. After five retention times the medium was changed to maltose-limitation (inducing conditions for the glaA promoter). Cultures were performed in triplicate. There was no apparent physiological difference between the two strains. Culture profiles and yield coefficients of all six cultures were almost identical. Mycelium obtained from fifth retention time on xylose and maltose was subjected to expression analysis using Affymetrix arrays with probe sets based on the A. niger genome. Among 14554 probe sets, glaA had highest expression-level on maltose. It was also expressed on xylose; but at a level four times lower than on maltose. ANOVA of triplicate expression results of four conditions (two strains and two C-sources) revealed that transcription of around 290 genes were >2-fold up- or down-regulated (p 4-fold). A single gene was identified as differentially expressed, when comparing the two strains. Interaction analysis showed this was independent of C-source. Expression of scFv did not result in increased UPR at steady state on maltose, however expression of some genes associated with UPR and protein secretion was enhanced (1.6-2-fold) on maltose compared to xylose. The investigation has yielded one new lead regarding the low yield production of heterologous proteins, and a number of clues on the differences in expression of genes involved metabolism of xylose and maltose.

Biochemistry and Secondary Metabolism

255. Chemogenomics of antioxidant inhibition of aflatoxin biosynthesis. Jong Kim[1], Jiujiang Yu[2,3], Noreen Mahoney[1], Kathleen Chan[1], Deepak Bhatnagar[2], Thomas Cleveland[2], Russell Molyneux[1], William Nierman[3,4] and Bruce Campbell[1*]. [1]Plant Mycotoxin Research, USDA, Albany, CA 94710 USA. bcc@pw.usda.gov. [2]Food and Feed Safety Research, USDA, New Orleans, LA 70124 USA. [3]The Institute of Genomic Research, Rockville, MD 20850 USA. [4]The George Washington University, Washington, DC 20006 USA.

Natural compounds were used to probe the functional genomics (chemogenomics) of aflatoxin biosynthesis. Caffeic acid (12mM) treatment of *Aspergillus flavus* inhibited aflatoxin biosynthesis without affecting growth. Microarray analysis showed genes in the aflatoxin biosynthetic cluster were completely down-regulated (log2 ratio –0.04 to –3.13). However, aflatoxin pathway regulator genes, *aflJ* or *laeA*, and sugar utilization cluster genes showed only minor changes in expression (log2 ratio 0.08 to –0.58). Genes in amino acid biosynthetic and aromatic compound metabolism were up-regulated (log2 ratio > 1.5). Most notable was up-regulation (log2 ratio 1.08 to 2.65, qRT-PCR) of four peroxiredoxin genes orthologous to the *AHP1* gene (alkyl hydroperoxide reductase) of *Saccharomyces cerevisiae*. Antioxidants trigger induction of *ahp* genes in *A. flavus* to protect the fungus from oxidizing agents, e.g., lipoperoxides, reactive oxygen species, etc. This detoxification attenuates upstream oxidative stress-response pathway signals, and suppresses aflatoxigenesis.

256. **BDM-1, a phosducin-like protein required for G-protein signaling in *Cryphonectria parasitica*, is a phosphoprotein targeted by casein kinase II**. Joanna Salamon-Kozubowska[1] and Angus L. Dawe [1,2]. [1]Department of Biology and [2]Molecular Biology Program, New Mexico State University, Las Cruces, NM 88003

G-protein signaling modulates many responses in fungi, including virulence of plant and animal pathogens. Previous studies with the plant pathogen *C. parasitica* have identified components that include a phosducin-like protein (BDM-1). Perturbation of the G-protein signaling pathways has been shown to affect virulence, pigmentation and sporulation. Deletion of the beta subunit CPGB-1 or BDM-1 affected these characteristics in an identical manner and caused similar alterations in transcriptional profile. G-proteins levels were reduced in accumulation following infection with virulence-attenuating hypoviruses. In this study we have shown by western blotting that CPGB-1 protein levels are undetectable in the absence of BDM-1 and by phosphatase treatment that BDM-1 exists in a phosphorylated form. Re- phosphylation of BDM-1 was accomplished in the presence of total protein lysates, but this activity was inhibited by a casein kinase II inhibitor, indicating that BDM-1 is a substrate for this kinase. With these tools it is now possible to explore the effects of phosphorylation on BDM-1 function and determine if this modification is altered by the presence of hypoviruses, thereby providing a model for exploring the molecular events associated with viral-mediated changes of cellular signaling pathways.

257. **Functional characterization of two cytochrome P450 monooxygenases, PaxP and PaxQ, involved in the biosynthesis of the indole-diterpene, paxilline.** Sanjay Saikia[1], Emily Parker[2,3] and Barry Scott[1]. [1]Institute of Molecular Biosciences and [2]Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand. [3]Current address: Department of Chemistry, Canterbury University, Christchurch, New Zealand.

Indole-diterpenes are a large, structurally and functionally diverse group of secondary metabolites produced by filamentous fungi. Biosynthetic schemes have been proposed for these metabolites but until recently none of the proposed steps had been validated by biochemical or genetic studies. Using *Penicillium paxilli* as a model experimental system to study indole-diterpene biosynthesis we previously showed by deletion analysis that a cluster of seven genes is required for paxilline biosynthesis. Two of these *pax* genes, *paxP* and *paxQ* (cytochrome P450 monooxygenases), are required in the later steps in this pathway. Here we describe the function of *paxP* and *paxQ* gene products by feeding proposed paxilline intermediates to strains lacking the *pax* cluster but containing ectopically integrated copies of *paxP* or *paxQ*. Transformants containing *paxP* converted paspaline into 13-desoxypaxilline as the major product and beta-PC-M6 as the minor product. beta-PC-M6, but not alpha-PC-M6, was also a substrate for PaxP and was converted to 13-desoxypaxilline. *paxQ*-containing transformants converted 13-desoxypaxilline into paxilline. These results confirm that paspaline, beta-PC-M6 and 13- desoxypaxilline are paxilline intermediates and that paspaline and beta-PC-M6 are substrates for PaxP, and 13- desoxypaxilline a substrate for PaxQ. PaxP and PaxQ also utilized beta-paxitriol and alpha-PC-M6 as substrates converting them to paxilline and alpha-paxitriol, respectively.

258. **LaeA, a methyltransferase regulating secondary metabolite production in *Aspergillus* spp.** Perrin, Robyn M; Bok, Jin Woo; Keller, Nancy P. 1630 Linden Dr., University of Wisconsin, Madison, WI USA 53706

Filamentous fungi are unique organisms – rivaled only by Actinomycetes and plants - in producing a wide range of secondary metabolites. These compounds often have obscure or unknown functions in the producing organism but have tremendous importance to humankind, displaying a broad range of useful pharmaceutical activities as well as less desirable phyto- and mycotoxic activities. The biosynthetic genes of most fungal secondary metabolites are clustered in genetic loci. LaeA is a nuclear methyltransferase that transcriptionally regulates secondary metabolite gene clusters in the filamentous fungal genus *Aspergillus*. Deletion and over- expression of *laeA* either silences or increases, respectively, the production of multiple secondary metabolites and expression of genes for their biosynthesis. Mutation of a canonical amino acid motif found in all methyltransferases results in a loss of function phenotype, indicating that methytransferase activity is critical for LaeA regulatory activity (Bok et al 2006 Mol Microbiol 61:1636). In order to understand the mechanism of LaeA function, we are using an *A. nidulans* strain expressing epitope-tagged LaeA and anti-LaeA antibodies to identify LaeA methylation targets and interacting proteins. Further analysis of biochemical function is being conducted using a series of mutated and truncated versions of recombinant LaeA. Elucidation of LaeA biochemical function is critical to understanding its role as a global regulator of secondary metabolism.

259. **Which "alphabet" do zygomycetes use for their chemical language?** Mareike Richter, Doreen Schachtschabel, Ute Neugebauer and Wilhelm Boland. Max Planck Institute for Chemical Ecology, Jena, Germany, mrichter@ice.mpg.de

During sexual interaction, zygomycete fungi communicate via carotene-derived compounds, namely trisporic acid and its precursors. The first sexually determined structures of *Mucor mucedo* are specialised aerial hyphae, called zygophores. Proteins are distributed uniformly in these hyphae. Carotenoids, in contrast, accumulate in the hyphal tip, shown by 2D Raman spectroscopy. The same technique also depicts the possible presence of trisporoids in the zygophore tips. The formation of zygophores is an important parameter to assess the bioactivity of trisporoids. Various synthetic trisporoid analogues exhibit a differential influence on zygophore formation in *Mucor mucedo*. Moreover, trisporoid production and carotene biosynthesis are linked via feedback regulation. The early precursor beta- carotene can be readily quantified by HPLC. After culture stimulation the correlation of morphological responses with beta-carotene concentration is allowed. The mechanistic basis of the induced regulation mechanisms remains to be established. The impact of trisporoids on gene transcription necessary for carotene synthesis and degradation is studied by real-time PCR. The early carotene biosynthesis is not under the control of trisporoids. But later enzymatic transformations, e.g. by phytoene desaturase and carotene dioxygenase respond to stimulation with synthetic trisporoids. In the future we want to investigate the consequences of different stimulating trisporoid analogues.

260. ***Agrobacterium tumefaciens*-mediated disruption of a polyketide synthase gene in *Cryphonectria parasitica*.** Keiichi Sudo[1], Heather L. McLane[2], Stuart B. Krasnoff[4], Sandra L. Anagnostakis[3], Donna M. Gibson[4], and Alice C.L. Churchill[1](acc7@cornell.edu). [1]Department of Plant Pathology, Cornell University, Ithaca, NY, [2]Boyce Thompson Institute, Ithaca, NY, [3]Connecticut Agricultural Experiment Station, New Haven, CT, [4]USDA-ARS, Plant Protection Research Unit, Ithaca, NY.

Cryphonectria parasitica is the causal agent of chestnut blight, which decimated American chestnut populations during the first half of the 20th century. Infection of the fungus with naturally occurring Hypovirus can reduce virulence, sporulation, and the production of fungal pigments, as well as perturbing signal transduction pathways. We cloned fragments of eight *C. parasitica* genes predicted to be involved in the synthesis of small molecule natural products. Most are polyketide synthase (PKS) genes,

which encode key enzymes for the synthesis of linear and aromatic polyketides. We identified and sequenced a ~6.0 kb gene, *PKS1*, which is a member of a predicted secondary metabolite gene cluster containing at least 13 other putative regulatory, modifying and efflux genes within a ~37 kb genomic region. The *PKS1* predicted protein is highly similar to several other uncharacterized fungal PKSs (E value of 0.0; greater than 60% identity). We disrupted *PKS1* in *C. parasitica* by *Agrobacterium tumefaciens*-mediated transformation and have begun characterization of the targeted gene knockout (KO) strains. There are no significant differences between the KO and ectopic control strains in colony growth rate on an agar medium or in Golden Delicious apple fruits, which are used as an alternate virulence assay. Virulence and mating assays on dormant chestnut stems are in progress, as are efforts to identify potential differences in development or nutritional requirements.

261. **Genetics, biosynthesis and evolution of the polyketide T-toxin produced by the fungus *Cochliobolus heterostrophus* race T**. Patrik Inderbitzin and B. Gillian Turgeon. Cornell University, Department of Plant Pathology, Ithaca, NY, USA. Emails: pri2@cornell.edu, bgt1@cornell.edu.

The polyketide virulence determinant, T-toxin, is produced by race T of the filamentous ascomycete *Cochliobolus heterostrophus*, the cause of Southern Corn Leaf Blight. The genetics, biosynthesis, and evolution of the ability to produce T-toxin differ significantly from those of other polyketides (PK). Unlike genes required for biosynthesis of the PKs, lovastatin and aflatoxin, for example, T-toxin genes are not clustered but are distributed at two loci encompassing ca. 1.2 Mb of AT rich, highly repetitive DNA present in race T but absent in non-T-toxin producing *C. heterostrophus* race O. Prior to this study, three genes were known to be required for T-toxin synthesis: two polyketide synthases (*PKS*) and the decarboxylase *DEC1*. We have identified six additional genes, all unique to race T. These include *LAM1*, *OXI1* and *HYD1*, encoding proteins similar to a 3-hydroxyacyl-CoA dehydrogenase, an oxidoreductase, and a serine hydrolase, respectively, and three genes encoding putative reductases, *RED1*, *RED2*, and *RED3*. Regarding function, some of the PKS reducing domains are degenerate and we speculate that enzymes encoded by the new genes assume these roles. Regarding gene distribution, T-toxin genes are not present in race O, in other *Cochliobolus* species, or in neighboring genera. An ortholog of one of the *PKS*s genes, however, is found in the corn pathogen, *Didymella zeae-maydis* and in the distantly related dung fungus, *Delitschia winteri*. This discontinuous distribution of the T-toxin genes, together with their physical location within AT rich, repetitive DNA at chromosomal breakpoints, suggests a complex evolutionary history.

262. **Identification of a compound that stimulates perithecial formation in *Giberella zeae.*** Seunghoon Lee[1], Jianming Jin[1], Sung-Hwan Yun[2], and Yin-Won Lee[1]. [1]School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea. [2]Dpartment of Biological Resources and Technology, Soonchunhyang University, Asan 336-745, Korea

Giberella zeae, a homothallic ascomycete, develops a fruiting body called perithecium during sexual stage. Many studies suggested that fungal sexual development is induced by specific changes in both gene expressions and metabolism. *G. zeae* is well known as a producer of many secondary metabolites such as mycotoxins and pigments. Recently, several genes required for production of metabolites such as polyketides and non-ribosomal peptides have been reported in this fungus; some are specifically related to perithecial development. To investigate the enhancement of sexual development by specific fungal metabolites, we searched and identify such compound from carrot agar culture of *G. zeae*. Exogenous supply of the purified compound to fungal culture caused increase in both the number of perithecia and black pigment production accumulated on the agar surface where peritheica were formed. NMR and MS analyses estimated that it is a long unsaturated compound with a formula $C_{38}H_{66}O_5$, Mw 604.57. Detailed characterization of the compound is in progress. Further studies will be performed to identify the genes responsible for the biosynthesis of this compound.

263. **Identification of AatB, a new component of the penicillin biosynthesis pathway of *Aspergillus nidulans*.** Petra Sproete [1], Michael J. Hynes [2], and Axel A. Brakhage [1]. [1] Leibniz Institute for Natural Product Research and Infection Biology (HKI) / Friedrich-Schiller- University Jena, Germany [2] Department of Genetics, University of Melbourne, Australia

The acyl coenzyme A:isopenicillin N acyltransferase (IAT) of *A. nidulans*, which is encoded by the *aatA* gene, catalyzes the final step of the penicillin biosynthesis, i.e., the exchange of the hydrophilic L-alpha-aminoadipic acid side chain of isopenicillin N for a hydrophobic acyl group. By analyzing a GFP-IAT protein fusion it could be shown that in *A. nidulans* – as in *Penicillium chrysogenum* – the IAT and therefore the enzymatic reaction is located in the peroxisomes. Further studies indicated a PTS1 dependent transport of the enzyme since the deletion of the rather untypical putative peroxisomal targeting sequence 1 (PTS1) Ala-Asn-Ile at the C terminus of the protein led to cytoplasmic localization of the IAT. Nevertheless, unlike the IAT of *P. chrysogenum*, such a mislocated enzyme seems to be functional because both, an *A. nidulans* strain lacking the PTS1 transporter and a strain possessing a mislocated IAT still produced about 50% and 80% of penicillin, respectively, compared to wild-type levels. Because an *aatA* disruption strain still was able to produce small amounts of an inhibitory substance, the *A. nidulans* database was searched for a putative redundant protein. A gene displaying a very similar exon distribution and a 58% similarity with the *aatA* gene but lacking the PTS1 encoding sequence was named *aatB*. First analyses of an *aatB* disruption strain indicated a participitation of AatB in penicillin biosynthesis of *A. nidulans*.

264. **Identification of all chitin synthase genes in *Rhizopus oryzae* genome.** Ayumi Abe and Teruo Sone. Research Faculty of Agriculture, Hokkaido University, Sapporo, JAPAN

Chitin and chitosan are main components of cell wall of zygomycetes. Chitin synthases in zygomycetes are thought to be the most important factor for the cell morphogenesis, but they have not been studied well. We aimed to identify all chitin syanthase genes in *R. oryzae* genome sequence data and to collect the information about the expression of those genes under the various culture conditions. We identified 26 chitin synthase genes from *R. oryzae* genome sequence, using conserved amino acid sequences by BLASTP analysis.

Phylogentic analysis resulted in the clustering of chitin synthases in zygomycetes independent from those of ascomycetes or basidiomycetes on which current classification is based. This indicates that a new classification of chitin synthases including the data of zygomycetes'

enzyme is appropriate. Notably, two genes in *R. oryzae* made a distinct clade, thus a new class of the enzyme, class VIII was proposed. Almost all of *R. oryzae* chitin synthase genes were expressed during at least one culture condition. Seven genes were specifically expressed during later period in solid culture, indicating the relationship to sporangiospore formation. These results may reflect the fact that zygomycetes' cell wall consists mainly of chitin and chitosan, and thus they conserved many isozymes of chitin synthases with different roles, in the genome.

265. **Analysis of the cyanase-like gene *cyn1* from the filamentous fungus *Sordaria macrospora.*** Skander Elleuche* and Stefanie Pöggeler. Department of Genetics of Eukaryotic Microorganisms - Institute of Microbiology and Genetics, Georg-August-University Göttingen, Germany

The enzyme cyanase has been found in several organisms such as plants, bacteria and archae. However, so far this enzyme that catalyzes the reaction of cyanate with bicarbonate to produce ammonia and carbon dioxide has not been characterized in fungi. In nature the substrate cyanate is formed from the spontaneous dissociation of urea in aqueous solution. Structural analysis of the *Escherichia coli* cyanase revealed that the enzyme is a homodecamer of 17 kDa subunits. Although cyanate at high concentrations can be fairly toxic to *E. coli*, cyanate can serve as the sole source of nitrogen for growth of *E. coli* as a result of cyanase-catalyzed decomposition to ammonia. In this study, we investigated the role of the cyanase-like gene *cyn1* from the filamentous ascomycete *Sordaria macrospora*. BLAST searches of a *S. macrospora* genomic sequence revealed a high degree of homology to cyanase encoding genes from various bacteria. The *S. macrospora cyn1* gene encodes a putative protein of 164 amino acids with a predicted molecular mass of 18.7 kD. Homologues of the *S. macrospora cyn1* gene were identified within the genomes of filamentous ascomycetes and basidiomycetes but not in the genomes of the yeasts *Saccharomyces cerevisiae* and Schizosaccharomyces pombe. To better understand the function of the *S. macrospora* cyanase, we generated a deltacyn1 gene disruption mutant. Analysis of the mutant strain, cellular localization of the cyanase and enzyme activity will be presented.

266. **An efficient gene targeting system to generate knockout mutants in the penicillin producer *Penicillium chrysogenum*.** Birgit Hoff and Ulrich Kück. Department of General and Molecular Botany, Ruhr-University Bochum, Universitaetsstr. 150, 44801 Bochum, Germany, birgit.hoff@rub.de

Penicillium chrysogenum is the main producer of the pharmaceutical relevant beta-lactam antibiotic penicillin. DNA-mediated transformation of this filamentous fungus results mostly in ectopic integrations of the molecules. For a functional analysis, it is however desirable to obtain gene substitutions by homologous recombination, a process which occurs with a rather low frequency in this fungus. A cellular feature that decreases homologous recombination is the non- homologous end-joining (NHEJ) pathway, a mechanism that involves the binding of the highly conserved Ku heterodimer at the ends of a DNA double-strand break. Integration events mediated by NHEJ do not rely on homologous sites and therefore attenuate homologous recombination, which results in decreased frequencies of a gene knockout in a given transformation experiment. To improve gene targeting efficiency in *P. chrysogenum*, we successfully deleted the *P. chrysogenum Pcku70* gene. No impairment in vegetative growth, sporulation and penicillin production could be assessed for the corresponding mutant strain but relative frequencies of homologous recombination were drastically increased to about 50- 80 % as deduced from targeting several different genes. To test the applicability of the *Pcku70* knockout strain, genes for global regulators were substituted by the phleomycin resistance gene. From the sum of our investigations, we can conclude that the *Pcku70* knockout strain is an ideal recipient for targeting of genes involved in regulation of secondary metabolism and fungal morphogenesis, and can therefore be useful in strain improvement programs.

267. **Regulation of genes coding for synthesis of beta-lactam antibiotics in *Acremonium chrysogenum* and *Penicillium chrysogenum*.** Birgit Hoff, Jacqueline Dreyer, and Ulrich Kück. Department of General and Molecular Botany, Ruhr-University Bochum, Universitaetsstr. 150, 44801 Bochum, Germany, birgit.hoff@rub.de

The filamentous fungi *Acremonium chrysogenum* and *Penicillium chrysogenum* are the main producer of the pharmaceutical relevant beta-lactam antibiotics cephalosporin C and penicillin. Expression of genes coding for the biosynthesis enzymes is known to be controlled by a complex network of global regulators such as CRE1, PACC, and CPCR1 etc. Using gene disruption approaches, we have generated knockout strains of both fungi to study the homologue of the *Aspergillus nidulans* velvet gene (*veA*). VeA co-ordinates asexual and sexual development in this homothallic fungus and was also found to control secondary metabolism. Expression and HPLC analyses have clearly indicated that the veA homologue acts as a regulator of beta-lactam biosynthesis in both fungi. In addition, detailed microscopic analyses have shown that deletion of *veA* caused alterations in hyphal morphology and fragmentation. From the sum of our investigations, we can conclude that the veA homologue in the two fungi controls both antibiotic biosynthesis and fungal morphogenesis.

268. **Use of the *DsRed* reporter gene to establish an RNAi system for the beta-lactam-antibiotic producer *Acremonium chrysogenum*.** Danielle Janus, Birgit Hoff, and Ulrich Kück. Department of General and Molecular Botany, Ruhr-University Bochum, Universitaetsstr. 150, 44801 Bochum, Germany, danielle.janus@rub.de

In filamentous fungi, RNA-silencing is an attractive alternative to disruption experiments for the functional analysis of genes. We have adapted the gene encoding the autofluorescent DsRed protein as a reporter to monitor the silencing process in fungal transformants. Using the cephalosporin C producer *Acremonium chrysogenum*, strains showing a high level expression of the *DsRed* gene were constructed, resulting in red-colored fungal colonies. Transfer of a hairpin-expressing vector carrying fragments of the *DsRed* gene allowed efficient silencing of *DsRed* expression. Monitoring of this process by northern hybridizations, real-time PCR, and spectrofluorometric measurements of the DsRed protein confirmed that downregulation of gene expression can be observed at different expression levels. The usefulness of this system was demonstrated by investigating co-silencing of *DsRed* together with *pcbC*, encoding the isopenicillin N synthase (IPNS), an enzyme involved in cephalosporin C biosynthesis. Downregulation of *pcbC* can easily be detected by a bioassay, measuring the antibiotic activity of individual strains. In addition, the presence of IPNS was investigated by western blot hybridization. All transformants having a colorless phenotype show a simultaneous downregulation of the *pcbC* gene. The RNA-silencing system presented here will be a powerful tool for strain improvement and genome-wide analysis of this biotechnically important filamentous fungus.

269. A novel gene cluster in *Fusarium graminearum* expressed under mycotoxin induction conditions. L. Harris[1], A. Saparno[1], N. Alexander[2], B. Blackwell[1], S. McCormick[2], D. Schneiderman[1], A. Desjardins[2], N. Tinker[1], J. Hattori[1], K. Seifert[1], T. Ouellet[1]. [1]Eastern Cereal & Oilseed Research Centre, Agriculture & Agri-Food Canada, Ottawa, ON, Canada; [2]Mycotoxin Research Unit, National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL. Email: harrislj@agr.gc.ca

We have identified a cluster of eight genes (gene loci *fg08077 – fg08084*) that is concomitantly up-regulated (Northern and qPCR analysis) under growth conditions that promote mycotoxin production. Proteomics experiments (iTRAQ analysis) have confirmed the up-regulation of proteins encoded by two of these genes under toxin induction conditions. Gene disruption and add-back experiments followed by metabolite analysis of the transformants indicated that one of the genes, *fg08079*, is directly involved in the synthesis of butenolide, a mycotoxin derived from glutamic acid. As expression of these genes can be detected very early during plant infection, butenolide may play a role in plant infection. However, greenhouse testing for FHB (Fusarium Head Blight) using disruption mutants of *fg08079* showed that this gene did not contribute significantly to virulence in wheat heads. The mycotoxin butenolide is produced by several *Fusarium* species and has been suggested to be associated with tall fescue toxicoses in grazing cattle. We are exploring interspecies conservation of this gene cluster by PCR screening and sequencing in other *Fusarium* genomes.

270. **Identification of a biosynthetic gene cluster for a secreted glycolipid with antifungal activity in *Ustilago maydis*.** Beate Teichmann, Lidan Liu and Michael Bölker. Department of Biology, University of Marburg, Karl-von-Frisch-Str. 8, D-35032 Marburg, Germany. E-mail: teichma3@staff.uni-marburg.de

Under conditions of nitrogen starvation, *U. maydis* secretes large amounts of the cellobiose lipid ustilagic acid. This amphiphatic glycolipid acts as biosurfactant and displays antibacterial and antifungal activity. Ustilagic acid consists of a cellobiose moiety O-glycosidically linked to the terminal hydroxyl group of di- or trihydroxypalmitic acid, respectively. We have identified a large gene cluster comprising 12 open reading frames that is responsible for UA biosynthesis. By mutational analysis and mass spectrometry we could characterize several enzymes that are involved in hydroxylation, glycosylation and secretion of UA. This allows us to propose a biosynthetic pathway for ustilagic acid. The cluster contains a putative Zn-finger DNA-binding protein, which is involved in regulation of cluster genes. We could show that expression of this transcriptional factor is necessary for UA production. Further we could demonstrate, that *U. maydis* has biocontrol activities against the plant pathogenic fungus *Botrytis cinerea*, which causes gray mold on tomato leaves. Coinoculation of haploid *U. maydis* cells with *B. cinerea* spores suppress all symptoms of disease. UA production is critical for the antagonistic activity of *U. maydis* cells against *B. cinerea* spores, since mutants defective for UA biosynthesis are unable to interfere with *B. cinerea* infection.

271. **Secondary metabolites: fundamental and niche-specific roles in fungal development and cell-cell interactions.** B. Gillian Turgeon and Shinichi Oide. Cornell University, New York

An exhaustive characterization of non-ribosomal peptide synthetase (*NPS*) genes of the corn pathogen, *Cochliobolus heterostrophus*, and the small peptides produced by the enzymes they encode, has been undertaken to ascertain what peptide secondary metabolite products are doing for the fungal cell. To date, the NRPS method of peptide biosynthesis has been described for filamentous ascomycetes (and to a limited extent, for basidiomycetes) and bacteria, only. NRPs have a broad spectrum of biological activities, many are useful in medicine, agriculture, industry, and biological research. Despite the fact that activities of the peptide products with respect to interactions with other organisms are well-documented, to suggest that this is their primary function is likely incorrect. For example, only one of the 12 *C. heterostrophus NPSs* is required for virulence. In fact, the physiological significance of these small peptides to the producing fungi is largely unknown. We document that NRPS enzymes are purveyors of small molecules for both basal metabolism and for specialized environmental niches. Certain *NPSs* are required for sexual reproduction, asexual reproduction, virulence, as nutrient 'gatherers' (such as iron-gathering siderophores), for responses to oxidative, nitrosative, nutrient, pH stress etc, for growth, and for hydrophobicity. Eight of the 12 *C. heterostrophus NPSs* are not conserved, while four are conserved in other ascomycetes. We have examined functional conservation of conserved genes in the wheat pathogen, *Fusarium graminearum*, and the Arabidopsis pathogen, *Alternaria brassicicola*. Comprehension of secondary metabolite function, from the perspective of the fungal cell itself, impacts our understanding of evolution of fungal pathogenicity mechanisms by addressing how pathogens become pathogens.

272. **CoA-Transferase from *Aspergillus nidulans* is required to forward the CoA-moiety from propionyl-CoA to acetate.** Christian Fleck, Matthias Brock. Leibniz Institute for Natural Product Research and Infection Biology e.V. – Hans-Knöll-Institute (HKI) christian.fleck@hki-jena.de

Metabolism of amino acids and propionate leads to the toxic intermediate propionyl-CoA. Efficient removal via the methylcitrate cycle is guaranteed by a functional methylcitrate synthase. Deletion of the coding region of this enzyme leads to the inability to grow on propionate or ethanol/propionate but not acetate/propionate. This led to the assumption that the level of propionyl-CoA may become reduced by direct transfer of the CoA-moiety to acetate. Therefore, we purified a CoA-transferase from *Aspergillus nidulans* and characterised the enzyme biochemically. The CoA-transferase was specific for the CoA-esters succinyl-CoA, propionyl-CoA, and acetyl-CoA as CoA-donors and the corresponding acids as acceptors. No other donors or acceptors suited as substrates. To elucidate the role of the enzyme under *in vivo* conditions the corresponding gene was deleted. The deletion mutant only showed mild phenotypes when tested for growth and development on propionate containing media. Therefore, a double mutant with a methylcitrate synthase deletion strain was created by sexual crossing. In contrast to the methylcitrate synthase deletion strain, the double mutant was neither able to grow on ethanol/propionate nor on acetate/propionate media. Thus, CoA-transferase is essential for removal of toxic propionyl-CoA in the presence of acetate as a CoA-acceptor.

273. **The aflatrem biosynthetic genes in *Aspergillus flavus* and *A. oryzae* are clustered in two separate regions of the genome.** Matthew J. Nicholson[1], Brendon J. Monahan[1], Bethan Pritchard[2], Gary A. Payne[2], and Barry Scott[1]. [1]Massey University, New Zealand. [2]North Carolina State University, USA.

Aflatrem is a potent tremorgenic toxin produced by *Aspergillus flavus* and is a member of a structurally diverse group of fungal secondary metabolites known as indole-diterpenes. Using *Penicillium paxilli* as a model experimental system for investigating indole-diterpene biosynthesis, we have shown that the products of four genes (*paxG, paxM, paxB* and *paxC*) are necessary for the production of paspaline, the first stable indole- diterpene intermediate, and that two P450 monooxygenases (encoded by *paxP* and paxQ) are required for the subsequent conversion of paspaline to paxilline. We previously identified a cluster of genes in *A. flavus* NRRL6541 proposed to be involved in aflatrem (*atm*) biosynthesis, that included homologues of *paxG* (*atmG*), *paxM* (*atmM*) and *paxC* (*atmC*) (Zhang *et al*., 2004). Here we report the identification of a homologous *atm* gene cluster in the sequenced genomes of *A. flavus* NRRL3357 and *A. oryzae* RIB40, and the identification of a second gene cluster, containing homologues of *paxB, paxP* and *paxQ* in these genomes. Comparative analysis of sequences from all three genomes revealed total syntenic conservation and high nucleotide identity both within and between genes. In both species the aflatrem biosynthetic genes are clustered in two discrete genomic regions. In *A. oryzae*, the first cluster (containing homologues of *paxG, paxC*, and *paxM*) is located subtelomerically on chromosome 7, whilst the second cluster (containing homologues of *paxB, paxP* and *paxQ*) is located on chromosome 5. Comparison with the paxilline gene cluster of *P. paxilli* provides important insights into the evolutionary history of these aflatrem gene clusters.

274. **Epigenetic regulation of the Aspergillus chemical arsenal.** Elliot Schwab and Nancy Keller. University of Wisconsin-Madison, Plant Pathology. Madison, WI.

Bioactive small molecules are critical in *Aspergillus* species during their development and interaction with other organisms. Genes dedicated to their production are encoded in clusters that can be located throughout the genome. We show that deletion of *hdaA*, an *Aspergillus nidulans* histone deacetylase (HDAC), causes transcriptional activation of two subtelomeric gene clusters -

and subsequent increased production of the corresponding molecules - but not of a telomere distal cluster. Furthermore, treatment of other fungal genera with HDAC inhibitors results in overproduction of several metabolites. The regulatory role of gene clustering and subtelomeric positioning of clusters will also be examined. Chromatin regulation of small molecule gene clusters may enable filamentous fungi to successfully exploit environmental resources by modifying chemical diversity.

275. **Characterization of a putative fusarin mycotoxin biosynthetic gene cluster in *Fusarium*.** Robert H. Proctor, Robert A.E. Butchko and Daren W. Brown. USDA-ARS National Center for Agricultural Utilization Research, Peoria, Illinois.

Fusarium verticillioides is a stalk and ear rot pathogen of maize and can produce the polyketide mycotoxins fumonisins in infected kernels. Although the genetics and biochemistry of fumonisin biosynthesis are relatively well understood in *F. verticillioides*, little is known about the biosynthesis of other secondary metabolites produced by this fungus and whether the regulatory mechanisms that affect fumonisin biosynthesis also affect biosynthesis of other secondary metabolites. To begin to address such issues, we are using microarray analysis to determine whether 15 previously identified *F. verticillioides* polyketide synthase (PKS) genes (Kroken et al, 2003 PNAS 100:15670- 15675) are located in clusters of coordinately regulated genes. To date, the analysis indicates that three of the PKS genes are located in clusters. For example, *PKS10*, which is required for the biosynthesis of fusarin mycotoxins, and eight contiguous genes adjacent to it exhibit similar changes in expression over time in liquid GYAM medium. These same nine genes also exhibit relatively high expression on whole maize kernels and low expression on embryo tissue. The predicted functions of some of these genes are consistent with enzymatic activities predicted to be required for fusarin biosynthesis. Similar arrangements of *PKS10* and at least some of the adjacent genes are present in the genomes of *F. graminearum* and *Nectria haematococca*. Together, the data suggest that *PKS10* and some of the genes next to it constitute a fusarin biosynthetic gene cluster.

276. **Characterization of a Fumonisin Biosynthetic Gene Cluster in *Fusarium oxysporum* strain O-1890.** Robert H. Proctor[1], Jeong-Ah Seo[2], Mark Busman[1], Yin-Won Lee[2] and Ronald Plattner[1]. [1] USDA-ARS National Center for Agricultural Utilization Research, Peoria, Illinois, USA. [2] Seoul National University, Seoul, Korea.

Fumonisins are carcinogenic mycotoxins produced by some *Fusarium* species. Most species, including *F. verticillioides*, produce predominantly B fumonisins (FBs), but *F. oxysporum* strain O-1890 produces predominantly C fumonisins (FCs). FBs have an alanine-derived methyl function that is absent in FCs. In this study, we determined the nucleotide sequence of the fumonisin biosynthetic gene (*FUM*) cluster in strain O-1890. Genes in the cluster were present in the same order and orientations as in the previously described *F. verticillioides* cluster. The *FUM* gene coding and intergenic regions were 89 – 92 % and 40 – 77% identical, respectively, in *F. oxysporum* and *F. verticillioides*. In contrast, the sequences flanking the cluster were completely different in the two species. As previously reported, the *F. verticillioides FUM* cluster gene *FUM8* encodes an oxoamine synthase and *fum8* mutants cannot produce fumonisins. In the current study, transformation of a *fum8* mutant with a wild-type *F. verticillioides FUM8* restored FB production while transformation with a *F. oxysporum* O-1890 *FUM8* resulted in FC production. These data indicate that the ability to produce FBs versus FCs is determined by different *FUM8* homologues.

277. **Functional analysis of the *Hypocrea jecorina lxr1* demonstrates that Lxr1 encodes a D-mannitol dehydrogenase and is not involved in L-arabinose catabolism.** Benjamin Metz, Stefan Polak, Christian P. Kubicek and Bernhard Seiboth. Research Area Gene Technology and Applied Biochemistry, Institute of Chemical Engineering, TU Wien, Austria.

The pentose L-arabinose is one of the main compounds of plant hemicelluloses and pectins. Most genes of the fungal L-arabinose pathway have been cloned for the industrial important fungi *H. jecorina* (anamorph: *Trichoderma reese*i). Cloning of an *H. jecorina*i L-xylulose reductase gene (*lxr1*) was reported previously by heterologous expression in *Saccharomyces cerevisiae* which enabled this yeast to grow on L-arabinose. A functional analysis of *lxr1* in our lab has now questioned the role of Lxr1 in L-arabinose catabolism: Growth of a Δ*lxr1* strain was not affected on L-arabinose and total L-xylulose reductase activity remained unchanged. In addition Northern analysis showed that *lxr1* was not L-arabinose inducible. In summary, our results clearly show that Lxr1 cannot be the enzyme which is active in the L-arabinose catabolic pathway. Our data suggest now that Lxr1 is a D-mannitol 2-dehydrogenase which is involved in developmental processes such as sporulation and germination. The polyol D-mannitol is accumumlated intracellularly by many fungal species and during spore germination D-mannitol is metabolized rapidly by D-mannitol 2-dehydrogenase. This is supported by our data that D-mannitol dehydrogenase activity was reduced in Δ*lxr1* strains and transcription levels were up-regulated during sporulation or germination.

278. **Search for genes involved in the neoechinulin A biosynthetic pathway in *Aspergillus* spp.** Masahiro Takeno, Momoko Horiuchi, Mayu Ohishi, Koji Kuramochi, Fumio Sugawara, and Takashi Kamakura. Tokyo University of Science, Noda, Japan.

Neoechinulin A is a member of the family of indole alkaloids comprised of tryptophane and alanine. It was isolated in 1973 from *Aspergillus rubber* by Yamazaki and co-workers. It was reported that the neoechinulin A was an antioxidant compound which suppresses lipid peroxidation in dried bonito flakes, Katsuobushi, and results of many studies suggested that Neoechinulin A

might be useful as a protectant against neuronal cell death in neurodegenerative diseases. Although the chemical synthesis method of neoechinulin A is already established, the biosynthetic pathway and genes involved in the biosynthesis of neoechinulin A are not determined yet. Therefore we have tried to find the genes by phage display method. Phage display method is one of the ways to find the binding protein(s) or peptide(s) to a particular compounds and at least some proteins involved in the neoechinulin A biosynthesis must have affinity with the neoechinulin A molecule. Since we expect that genes for biosysthesis of neoechinulin A may form gene cluster(s) in the fungal genome similar to other gene clusters for secondary metabolites, the gene isolated by phage display could be the guide for us to access the gene cluster for neoechinulin A biosynthesis. As a result, we found a candidate of biosynthesis gene of neoechinulin A. The candidate had homologous sequence with non-ribosomal peptide synthetase (NRPS).

279. **Sweet 'n' greasy: glycolipid biosynthetic gene clusters from *Ustilago maydis***. Sandra Hewald, Beate Teichmann, Lidan Liu, Uwe Linne and Michael Bölker. University of Marburg, Dept. of Biology, Karl-von Frisch-Strasse 8, D-35032 Marburg, Germany. e-mail: boelker@staff.uni-marburg.de

Under conditions of nitrogen starvation, *Ustilago maydis* produces two classes of extracellular glycolipids that act as biosurfactants. Ustilagic acid (UA) consists of a cellobiose moiety glycosidically linked to the omega-hydroxyl group of 15,16-dihydroxypalmitic acid. In addition, *U. maydis* cells secrete mannosylerythritol lipids (MELs), which consist of the disaccharide mannosylerythritol esterified with acyl and acetyl groups at the mannosyl group. We have identified two large gene clusters, which are responsible for extracellular glycolipid production. Database comparisons and mutational analysis allowed us to propose biosynthetic pathways for both of these unusual secondary metabolites. Since *U. maydis* is very amenable to genetic analysis this organism can be used for metabolic engineering of these extracellular glycolipids. Deletion of a MEL-specific acetyl transferase resulted in the production of deacetylated derivatives of this glycolipid. By targeted disruption of UA-specific hydroxylases we were able to create strains that secrete novel variants of ustilagic acid.

280. **Integrated database for functional analysis in *Aspergillus flavus*.** C. P. Smith[1], C. P. Woloshuk[2], N. P. Keller[3], J. Yu[4], and G. A. Payne[5]. [1]North Carolina State University, Raleigh, USA. chris@statgen.ncsu.edu. [2] Purdue University, West Lafayette, IN, USA. woloshuk@purdue.edu. [3]University of Wisconsin, Madison, WI, USA. npk@plantpath.wisc.edu. [4]USDA/ARS/SRRC, New Orleans, LA, USA. jiuyu@srrc.ars.usda.gov. [5]North Carolina State University, Raleigh, USA. gary_payne@ncsu.edu.

Aspergillus flavus is a plant and animal pathogen that also produces the carcinogen, aflatoxin. Because of its economic importance and well characterized pathway of aflatoxin biosynthesis, several labs are studying the development, metabolism, ecology, and pathogenicity of this fungus. To facilitate the research efforts in these areas and to identify potential genes and pathways for functional analysis, we are developing a database to integrate multiple categories of data. This database resource will serve two important functions: 1) it will provide a platform for the deposition of data from individual experiments; and 2) it will permit the ready analysis of composite data from all experiments enabling researchers to mine a larger data set. It will include phenotypic measurements, gene expression data from microarrays, and metabolic profile information and will be flexible enough to allow the addition of new types of measurement in the future. Users will interact with the database through a web based interface and will be able to: describe experiments; upload data gathered during those experiments; run analyses on the data; select and download raw data; select and download the results of analyses. Recently acquired lab and field data will be used to highlight the structure and utility of this database. These measurements will include fungal growth, aflatoxin concentrations, and gene expression data acquired from lab studies and infected developing maize seeds. This research was funded by USDA/NRI/CGP 2006-35604-16666.

281. **Analysis of Methylene Tetrahydrofolate Reductases in filamentous fungi.** Rasmus J.N. Frandsen and Henriette Giese, Department of Ecology, Section for Gen and Micro, Copenhagen University, Denmark. raf@kvl.dk and heg@kvl.dk

A. tumefaciens mediated random mutagenesis of *Fusarium pseudograminearum* led to the isolation of a mutant which accumulated rubrofusarin instead of the normal mycelium pigment, aurofusarin. Determination of the T- DNA integration site and a blast analysis against *F. graminearum* showed that a gene (Fg-met13) with homology to methylene tetrahydrofolate reductases (MTHR) was affected. Analysis of all fully sequenced fungi showed that they all had two MTHR genes. Multiple sequence alignment of the identified Met12 and Met13 sequences shows that the sequences clustered in two groups, suggesting distinct evolutionary histories and functions. Deletion of met12 and met13 in *S. cerevisiae* have earlier showed that Sc-met13 is required for methionine biosynthesis, while no function or phenotype have been assigned to Sc-met12. Targeted replacement of Fg-met12 and Fg- met13 showed that Fg-met12, and not as expected Fg-met13, is required for methionine biosynthesis in *Fusarium*. Complementation of the Sc-met13 deletion strain with Fg-met12 and Fg-met13 showed that both are functional MTHRs. We are currently testing the expression patterns to see if this can account for the differences between the two species. The situation in other fungi is also under investigation. We are currently exploring the possibility that Fg-met13 is indirectly involved in aurofusarin formation, possibly by synthesising a co-factor required by one of the enzymes known to participate in the pathway.

282. ***FvVE1* Differentially Regulates the Biosynthesis of Fumonisins and Bikaverin in *Fusarium verticillioides*.** Kyung Myung [1], Shaojie Li[1], Hamed K. Abbas[2] and Ana M. Calvo[1]. [1]Department of Biological Sciences, Northern Illinois University, 1425 W Lincoln Hwy., Dekalb, Illinois. 60115, USA. [2]Crop Genetics and Production Research Unit, USDA-ARS, Stoneville, Mississippi 38766, USA.

The *veA* homologous genes positively regulate sterigmatocystin and aflatoxin production in *Aspergillus nidulans* and in *Aspergillus parasiticus* and *Aspergillus flavus*, respectively. Whether *veA* homologs have a role in regulating secondary metabolism in other fungal genera is unknown. In this study, we examined the role of the *veA* homologous gene *FvVE1* on the production of two different types of toxins in the plant pathogen *Fusarium verticillioides*. We found that deletion of *FvVE1* completely suppressed fumonisin production on two natural substrates, corn and rice, but did not completely eliminate fumonisin production in fumonisin-inducing complex liquid medium GYAM. In contrast, deletion of *FvVE1* reduced the yield of bikaverin in GYAM while it increased bikaverin production in corn. The effects of *FvVE1* deletion on toxin production were found to be the same in the two different mating types. Complemented strains restored the production of both fumonisins and bikaverin to wild-type levels. Our results strongly suggest that *FvVE1* differentially regulates fumonisin and bikaverin biosynthesis in a medium-dependent manner.

283. Characterization of putative protein phosphatase gene in *Fusarium verticillioides* that is associated with fumonisin biosynthesis, cellular development, and conidiation. **Yoon-E Choi and Won-Bo Shim, Dept. Plant Pathology and Microbiology, Texas A&M University, TX 77843-2132**

***Fusarium verticillioides* is known to cause maize ear rot and produce fumonisin B1 (FB1). To date, our understanding of the regulatory mechanisms associated with FB1 biosynthesis is incomplete. In this study, we present the isolation and characterization of a putative protein phosphatase gene (*CPP1*) in *F. verticillioides* and its role in FB1 biosynthesis. *CPP1* expression study suggested that *CPP1* is associated with FB1 biosynthesis in a negative manner. *CPP1* knock-out mutants were generated, and we select a mutant strain PP179 for further analysis. PP179 showed elevated expression of FB1 biosynthetic genes, including *FUM1* (PKS gene), and in turn produced a higher level of FB1 than the wild-type progenitor. Other significant PP179 phenotypes included reduced radial growth on solid media, reduced conidia germination efficiency, increased macroconidia development, and hyphal swelling. To verify that these phenotypes were directly due to *CPP1* deletion, we complemented PP179 with the *CPP1* gene. The complement strain, PPC4, showed the wild-type level *FUM1* expression and restored the FB1 production level to that of wild type, providing molecular evidence that *CPP1* is negatively associated with FB1 biosynthesis. Phenotypes such as growth defect, macroconidia production, and hyphal swelling were also restored in PPC4. The results suggest that *CPP1* is a negative regulator of FB1 biosynthesis, however, the pleotropic effects of *CPP1* deletion precluded definitive conclusions. Further study is necessary to identify a signaling pathway downstream of *CPP1* that is directly associated with FB1 biosynthesis in *F. verticillioides*.**

284. Investigating polyketide production in fungi: Tenellin and other PKS/NRPS genes. **Andy Bailey, Kirstin Eley, Laura Halo, Thomas Thomas, Kate Harley, Deirdre Hurley, Zongshu Song, Russell Cox, Colin Lazarus and Thomas Simpson. School of Biological Sciences and School of Chemistry, University of Bristol, Bristol, UK.**

Type I Polyketide Synthases (PKS) are large multidomain, multifunctional enzymes. In Fungi, these enzymes are iterative, but how they are programmed is mysterious, meaning that it is impossible to predict the chemical made even if the gene sequence has been determined. Using class-specific degenerate PCR-primers, we have isolated fragments from several PKS and PKS/NRPS hybrid genes from different fungi. We have sequenced the gene-cluster responsible for synthesis of the PKS/NRPS Tenellin produced by the insect pathogen *Beauveria bassiana*. Confirmation of the identity of this cluster was achieved by targeted disruption of the PKS/NRPS, with knockout strains failing to produce Tenellin. The gene shows the expected architecture with KS-AT-DH-cMeT-KR-ACP for the polyketide component, followed by a single NRPS domain including the C-A-T&R domains required for addition of the aminoacid moiety. The disrupted strain was still fully virulent on the insect host, showing that tenellin is not essential for the disease process. We hope to gain more understanding about how PKS proteins are programmed to make their specific product, particularly how the number of extension cycles is regulated and how the levels of reduction are controlled, by using domain-swaps between different PKS genes. These are expressed in *Aspergillus oryzae* and coupled with chemical analysis of any resulting new secondary metabolites.

285. Site-Directed Mutagenesis Studies of Pheromones of *Schizophyllum commune*. **James L. Mackey and Thomas J. Fowler, Southern Illinois University Edwardsville, Edwardsville, Illinois. tfowler@siue.edu**

Mating of homokaryons and maintenance of the dikaryon in *S. commune* are controlled in part by pheromone- responsive G protein-coupled receptors (GPCRs) and short, lipopeptide pheromones. Pheromone receptor activation initiates a

presumed signal transduction cascade that leads to nuclear migration and fusion of hook cells. Pheromones from one subset of *S. commune* mating pheromones have a central cluster of three amino acids that vary between the pheromones. These pheromones can activate more than one receptor and these sets of receptors differ for each pheromone studied, but the sets have some receptors in common. Site-directed mutagenesis experiments focused on changing these three amino acids. Signal initiation was studied by heterologous expression of pheromones and receptors in *Saccharomyces cerevisiae*. Single amino acids substitutions have shown no change in receptor specificity for the four pheromones studied in this group. This is different compared to previous studies of a different subset of *S. commune* mating pheromones where single amino acid residues controlled the specificity of the pheromone. The current data suggest that this subset of pheromones does not rely on single amino acid residues for specificity of interaction with receptors. Double and triple amino acid substitutions should determine the key to specificity for this pheromone group. This research is funded by NSF.

286. Withdrawn

287. *AREA* in *Fusarium verticillioides* Affects Growth and Fumonisin Biosynthesis In Colonized Maize Kernels. Hun Kim and C. P. Woloshuk. Purdue University, West Lafayette, IN. hunkim@purdue.edu

Fumonisins are nitrogen-containing, secondary metabolites produced by *Fusarium verticillioides*. Evidence indicate that regulation of fumonisin biosynthesis is governed by carbon source, nitrogen source and pH. Previous studies have indicated that fumonisin B1 (FB1) biosynthesis is repressed in cultures containing ammonium phosphate (AP) as the nitrogen source. The fungus also fails to produce FB1 when grown on blister kernels, the earliest stages of kernel development. We generated a strain of *F. verticillioides* with a disrupted *AREA* gene, a regulator of nitrogen metabolism. The growth rate of the *areA* mutant on defined media containing either AP or casein hydrolysate was similar to the wild type. In contrast, the mutant grew poorly on media containing a nitrogen source of sodium nitrate or BSA. When the mutant was grown on mature maize kernels, it did not produce FB1, most likely because of poor growth. When AP was added to kernels, the mutant grew as well as the wild-type strain but neither strain accumulated FB1. This result indicates that the *AREA* gene is needed for growth on mature kernels and possibly for *FUM* gene expression. The *areA* mutant also grew well on blister kernels indicating that the elevated levels of free amino acids known to be present in early kernel development are sufficient to support growth of the mutant. We will present results from additional experiments that demonstrate how different nitrogen and carbon sources as well as pH affect FB1 biosynthesis in a strain that over-expresses *AREA*.

288. Comparative analysis of secondary metabolism gene clusters from two strains of *Aspergillus fumigatus* and closely related species *Neosartorya fischeri* and *Aspergillus clavatus*. Natalie Fedorova[1], Vinita Joardar[1], Jonathan Crabtree[1], Rama Maiti[1], Paolo Amedeo[1], David Denning[2], Jennifer Wortman[1], Geoffrey Turner[3], and William Nierman[1]. [1]The Institute for Genomic Research, Rockville, MD, USA; [2]School of Medicine and Faculty of Life Sciences, The University of Manchester, Manchester, UK; [3]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK. Email: natalief@tigr.org

Genes responsible for biosynthesis of secondary metabolites are often referred to as the most variable segment of fungal genomes. Comparative analysis of secondary metabolism gene clusters from *A. fumigatus* and the two closely related species confirmed that these organisms are indeed relentlessly updating their repertoire of mycotoxins. Out of 23 clusters identified in *A. fumigatus*, only seven have putative orthologs in the other two aspergilli. Most of these 'core' clusters are highly conserved and appear to be under strong negative selection. The rest of the clusters, however, are species- or even strain-specific, implying that the aspergilli must utilize specific mechanisms to ensure a quick divergence and turnover of clusters in the course of evolution. These mechanisms may involve *de-novo* assembly, segmental duplication, translocation, accelerated differentiation, and differential loss of clusters in various lineages.

289. Impact of methionine synthase deletion on metabolism of the phytopathogenic fungus *Magnaporthe grisea*. Oceane Frelin, Marie Emmanuelle Saint-macary, Marie Joseph Gagey, Christelle Barbisan, Roland Beffa, Marc-henri Lebrun, Geraldine Mey and Michel Droux. UMR2847 CNRS/Bayer CropScience, Physiologie des plantes et des champignons lors de l'infection, Bayer CropScience, 14-20 rue Pierre Baizet 69009 LYON

In organisms capable of sulfate assimilation, the reduced sulfur atom is incorporated in organic molecules to generate the two sulfur amino acids, cysteine and methionine. In filamentous fungi their biosynthetic pathways consist in features reported in both plant and bacteria as well as those described in the yeast *Saccharomyces cerevisiae*. Studies on sulfur metabolism in the phytopathogenic fungus *Magnaporthe grisea* were developed to understand its role during fungal development. With this aim a mutant of the methionine synthase (Met6) was obtained using gene replacement. The *met6* null mutants were auxotrophic for methionine, reduced in sporulation and were unable to penetrate into host leaves. Our goal is to further understand the impact of absence of the methionine synthesis step on general metabolism in *M. grisea*. We performed targeted metabolite analysis through reversed chromatography using HPLC. In parallel, a targeted gene

expression analysis through qPCR was also realized. Results show drastic differences in the sulfur containing compounds, amino acid profiles and sulfur-linked gene expression level between wild type strain and the *met6* null mutants. Results indicate that lack of methionine synthase induces both complex metabolic and transcriptomic perturbations

290. Phylogenomic analysis of fungal nonribosomal peptide synthetases reveals modular duplication, loss, and shuffling as innovative forces. Scott Kroken[1], B. Gillian Turgeon[2]. [1]Division of Plant Pathology and Microbiology, Dept. of Plant Pathology and Microbiology. University of Arizona. kroken@ag.arizona.edu [2]Dept. of Plant Pathology, Cornell University. bgt1@cornell.edu

The majority of secondary metabolism in euascomycete fungi is based on a diversity of various and variously modified nonribosomal peptides, polyketides, and hybrids of these two backbone molecules. The evolutionary history of nonribosomal peptide synthetases (NPSs) is similar to that reported for polyketide synthetases (Kroken et al 2003): fungi and bacteria have separate clades of NPSs (with some notable exceptions of horizontal transfer), rampant duplication and divergence, discontinuous distributions of orthologous NPSs, and a rapid drop-off of shared orthologs with increasing phylogenetic distance among fungal species. In addition, the multi-modular construction of NPSs adds another dimension to their evolution. Orthologous NPSs, which are often found in orthologous gene clusters, frequently vary in the presence/absence of certain domains. Paralagous NPSs are the result of modular duplication, and subsequent concatenation or rearrangement of those modules. Rearrangement is also found at the domain level, as the adenylation and condensation domains of each module often have different evolutionary histories. Therefore, an innovative factor of NPS evolution is the reordering of adenylation domains that specify different amino acids, and condensation domains that appear to specify how the amino acid is to be covalently bound in the nascent oligopeptide. For example, the intermediate and terminal condensation domain of fungal NPSs form separate clades, suggesting that the terminal condensation domain also functions as a peptide release factor. Fungal-type NPSs do not have, or apparently need, a thioesterase domain as found in bacterial-type NPSs (which include the ACV synthetases used for penicillin synthesis).

Gene Regulation

291. The Protein Kinase Tor1 Links Nutrient Sensing and Filamentous Growth in *Candida albicans*. Robert J. Bastidas, Maria Cardenas-Corona and Joseph Heitman. Department of Molecular Genetics and Microbiology. Duke University. rjb10@duke.edu

The life cycle of *Candida albicans* consists of two phases, a yeast phase and a filamentous phase. The transition between yeast and filamentous growth is controlled by a variety of signaling networks. In eukaryotic cells, the protein kinase Tor (Target of rapamycin) functions in a signaling pathway implicated in regulating cellular responses to nutrients that is conserved from yeast to humans. In this work, we have begun to characterize the function of *C.albicans* Tor1. Using rapamycin, a pharmacological inhibitor of Tor, we find that CaTor1 is necessary for the ability of *C.albicans* cells to switch between yeast and filamentous states. First, exposure to rapamycin under non- filamentation inducing conditions elicits differential expression of genes involved in filamentation, as detected by microarray analysis. Second, northern analysis revealed that CaTor1 is required for the repression of hyphal specific gene induction. Third, inhibition of CaTor1 enhances germ tube formation during growth on filamentation inducing conditions. Finally, a screen of transcription factor mutants identified mutations in the filamentous growth repressors CaNrg1 and CaTup1 that result in rapamycin hypersensitivity. Our results support a model in which CaTor1 couples nutrient sensing signaling cascades and filamentous growth possibly by governing the activity of CaTup1 and CaNrg1.

292. Light responses in *Mucor circinelloides*: a regulatory network involving three *white collar-1* genes and a RING-finger protein. Santiago Torres-Martínez, Fátima Silva, and Victoriano Garre. Department of Genetics and Microbiology, Faculty of Biology. University of Murcia. Murcia. Spain

Light regulates many developmental and physiological processes in a large number of organisms, including fungi. The best- known light response in the fungus *Mucor circinelloides* is the biosynthesis of beta-carotene, although we have recently demonstrated that its sporangiophores also respond to light, exhibiting a positive phototropism. Analysis of both responses to different light wavelengths within the visible spectrum demonstrated that phototropism is induced by green and blue light, whereas carotenogenesis is only induced by blue light. We have identified in *M. circinelloides* three *white collar-1* genes (*mcwc-1a, mcwc-1b* and *mcwc-1c*) coding for proteins showing similarity with the WC-1 photoreceptor of *Neurospora crassa*. All three contain a LOV (light, oxygen or voltage) domain, similar to that present in fungal and plant blue-light receptors. Knockout mutants for each *mcwc-1* gene were generated to characterize gene functions. Only *mcwc-1c* mutants were defective in light induction of carotene biosynthesis, indicating that *mcwc-1c* is involved in the light transduction pathway that control carotenogenesis. We have also shown that positive phototropism is controlled by the *mcwc-1a* gene. It seems therefore that *mcwc-1a* and *mcwc-1c* genes control different light transduction pathways. Cross-

talk between both pathways probably exists because lack of *mcwc-1a* function clearly affects light regulation of *mcwc-1c* expression. Studies of interactions between *mcwc-1* genes and *crgA* gene, a RING-finger repressor of photocarotenogenesis in *M. circinelloides*, revealed that *crgA* function in carotenogenesis is mediated by *mcwc-1b* gene, since mutations in this gene suppresses the constitutive phenotype for carotenogenesis showed by the *crgA* mutants. These results confirm the complex network of pathways controlling the light responses in *M. circinelloides*.

293. Enhanced Responsiveness and Sensitivity to Blue Light by *blr*-2 Overexpression in *Trichoderma atroviride.* Ulises Esquivel-Naranjo and Alfredo Herrera-Estrella Departamento de Ingeniería Genética y Laboratorio Nacional de Genómica para la Biodiversidad, Cinvestav Campus Guanajuato, Apartado postal 629, Irapuato 36500, México. Email: aherrera@ira.cinvestav.mx

Light is an environmental factor that regulates pivotal processes in living organisms, such as growth, development, reproduction and metabolism. Blue light activates asexual reproduction in *T. atroviride* through the transcription factors BLR-1 and BLR-2 that regulate the light responsive genes. BLR proteins contain PAS and GATA-type DNA binding domains and both proteins are essential for the regulation of blue light responsive genes and photoconidiation. In this work, we show that BLR-2 is a limiting factor for photo-perception. Overexpression of *blr*-2 resulted in increased photoconidiation and a stronger expression of light induced genes. The characterization of light responses in *blr*-2 overexpressers allowed us to identify a crosstalk between blue and red light input systems and an interaction of the BLR proteins with other(s) light perception system(s) in *T. atroviride*. *blr*-2 overexpression caused a marked reduction of growth when the fungus was grown under defined photoperiods, including a period of strong sensitivity to light, followed by a period of insensitivity. Long periods of incubation under this condition permitted recovery of a rhythmic growth similar to that of the wild type. A novel light dependent role for the BLR proteins was found in the positive posttranscriptional regulation of *blr*-2 expression, in addition to a transcriptional role, which occurred even in the dark. Finally, we demonstrated that *blr*-2 overexpression caused higher sensitivity to blue light and therefore propose that the pre-formation of BLR-1/BLR-2 complexes is key for adequate light perception in *T. atroviride*.

294. Molecular Characterization of Ligth Responses in the Celullolitic Fungus *Trichoderma reesei*. F.X. Castellanos-Juarez, A. Sánchez, M. Schmoll, C. Kubicek, and A. Herrera-Estrella. Laboratorio Nacional de Genómica para la Biodiversidad, Cinvestav Campus Guanajuato, AP 629, Irapuato, Guanajuato, México 36500 aherrera@ira.cinvestav.mx

Sunlight is a key environmental factor that determines the behaviour of living organisms. Among the diversity of responses triggered by light, in fungi important efforts have been directed to studying photomorphogenesis, phototropism, pigmentation and circadian rhythms. In *Trichoderma atroviride*, conidiation is activated by blue light. Two genes *(blr1/blr2)* homologues of the *Neurospora crassa wc1/wc2* are essential for photoconidiation and regulation of blue light responsive genes. Recently, *envoy*, a gene homologous to the *N. crassa vivid* has been identified in *T. reesei, vivid* encodes a secondary blue-light receptor, necessary for adaptation to constant illumination and to detect changes in light intensity. Although *envoy* was unable to complement the wild type phenotype of a *vivid* mutant, this gene is necessary for light tolerance. In the present work, we analyzed the role of Envoy in photoadaptation, to detect changes in light intensity and its regulation through BLR perception system. Under constant illumination we found Envoy is required to turn off the expression of blue light responsive genes similar to VIVID. This result is consistent with its contribution to the light tolerance during mycelial growth. On the other hand, we observed that an *envoy* mutant was unable to detect changes in light intensity. In order to scrutinize the participation of BLR proteins in the *envoy* regulation, we generated mutants in both genes and their characterization showed a lack of photoconidiation and regulation of the blue light responsive genes including *envoy*.

295. Transcriptional controls of carbon source utilization in *Aspergillus nidulans*. Michael Hynes. Department of Genetics, University of Melbourne, Parkville, Australia.

In *Aspergillus nidulans*, acetate utilisation involves acetyl-CoA formation in the cytoplasm and metabolism via the glyoxalate cycle in peroxisomes and via the TCA cycle in mitochondria. A cytoplasmic carnitine-acetyl transferase allows acetyl-CoA to enter the organelles as acetyl-carnitine which is converted back to acetyl-CoA by a second carnitine-acetyl transferase encoded by *acuJ*, a gene required for growth on both acetate and fatty acids which are converted to acetyl-CoA by peroxisomal beta-oxidation. A peroxisomal targetting mutation results in loss of growth on fatty acids indicating that AcuJ is necessary for transport of acetyl-CoA from peroxisomes to the mitochondria. Growth on acetate is not affected, indicating that acetyl-CoA can be metabolised via the glyoxalate cycle in the cytoplasm. This is supported by the ability of various peroxisome mutants to grow on acetate but not on fatty acids. There are three classes of genes - those induced by acetate only; those induced by both acetate and fatty acids including *acuJ* and genes for the glyoxalate cycle and a very large number induced only by fatty acids including genes encoding beta-oxidation enzymes and peroxins. The transcription factor FacB, specific for acetate induction, is regulated by glucose repression and induction by acetyl-carnitine. FarA, FarB (orthologs of the highly conserved cutinase transcription factors) and ScfA, are required for fatty

acid induction. FarA orthologs are also found in the hemi-ascomycetes, *Candida albicans*, *Debaryomyces hansenii* and *Yarrowia lipolytica*. Carbon sources metabolised via the TCA cycle, like acetate and fatty acids, require gluconeogenesis. Two transcription factors are required for induction of relevant enzymes in response to either malate or oxaloacetate.

296. Carbon sensing regulation of G protein signal pathway in *Neurospora crassa*. Liande Li and Katherine Borkovich. University of California, Riverside.

***N. crassa* is an excellent model to study heterotrimeric G protein signaling pathways, as *N. crassa* can grow in diverse environments, and one of the major systems used by *N. crassa* to sense extracellular changes involves heterotrimeric G proteins. This study has focused on examining the carbon sensing pathways involving GPR-4, a predicted G protein coupled receptor (GPCR) of *N. crassa*, and regulated G proteins. Since GPR-4 related GPCRs are found in the genomes of many fungal pathogens, study of GPR-4 may shed light on the functions of these proteins during pathogenesis, growth and development. Our results support the hypothesis that GPR-4 is coupled to GNA-1 in a cAMP signaling pathway that regulates the response to carbon sources in *N. crassa*. delta *gpr-4* mutants have severe defects on glycerol medium suggested that the GPR-4 signaling pathway may be involved in glycerol utilization, but Northern analyses showed that deletion of *gpr-4* does not greatly influence expression of many genes implicated in glycerol utilization. delta *gpr-4* strains also lack the transient increase in cAMP levels observed in wild type after addition of glucose to glycerol-grown cultures. In order to identify downstream signaling pathways controlled by GPR-4 during carbon sensing, microarray analyses are being performed using samples from wild type and *gpr-4* and *gna-1* mutants cultured with different carbon sources. The regulated genes will yield information about G protein regulation of carbon sensing in filamentous fungi.**

297. Gene silencing in *Mucor circinelloides* by expression of hairpin RNAs: Role of the *dcl-2* and *rdrp* genes. Rosa M. Ruiz-Vazquez, Juan P. de Haro, Silvia Calo and Santiago Torres-Martinez. Department of Genetics and Micro, Faculty of Biology, University of Murcia, Murcia 30071, Spain.

We have previously demonstrated the existence of a gene silencing mechanism triggered by self-replicative transgenes in the zygomycete *Mucor circinelloides*. Gene silencing is associated with two size classes of siRNA, 25-nt and 21-nt long, which are differentially accumulated through the vegetative growth of the silenced strains. These two classes of siRNAs are preferentially produced from the 3'-end of the endogenous gene and both are associated with amplification and spreading of the silencing signal. To increase the silencing frequency and the stability of the silencing effects during vegetative growth, we have developed hairpin expressing plasmids designed to silence an endogenous gene. Hairpin constructs containing an intron are highly efficient silencing triggers in *M. circinelloides*, the expression of hairpin RNA being associated with the accumulation of the two size classes of siRNA. We have isolated two *dicer*-like genes of *M. circinelloides*. Phenotypic analysis of null *dcl-1* mutants demonstrated that the *dcl-1* gene is not essential for sense transgene or dsRNA-induced gene silencing, since *dcl-1* mutants are able to silence gene expression and to produce the two classes of siRNA. However, null *dcl-2* mutants are severely impaired in gene silencing, revealing an essential role of this gene in RNA silencing and suggesting that a single Dicer enzyme is responsible for the production of the two size classes of siRNAs. We have also isolated a *M. circinelloides rdrp* gene, which codes for a RNA-dependent RNA polymerase. The role of the *rdrp* gene in gene silencing triggered by sense transgenes or dsRNA, as deduced from the phenotype of null *rdrp* mutants, will be presented.

298. RNA expression in *Cryptococcus neoformans* during pulmonary cryptococcosis. Guanggan Hu, A Sham and JW Kronstad (Michael Smith Laboratories, The University of British Columbia, Vancouver, BC, Canada)

***C. neoformans* is an encapsulated fungus that causes meningoencephalitis in immunocompromised individuals, and that is believed to be a facultative intracellular pathogen during early stages of pulmonary infection. Alveolar macrophages are the major phagocytic cells in the murine lung and the percentage of Cryptococcus cells inside alveolar macrophages is known to reach a maximum at 8 h after infection, while most of the yeast cells are extracellular by 24 h. Although the course of infection is relatively clear, information on *C. neoformans* gene expression during infection is quite limited. The goal of the present study was to determine the early transcriptome changes that occur in *C. neoformans* during pulmonary cryptococcosis. To achieve this goal, we performed long SAGE (serial analysis of gene expression) analysis on *C. neoformans* cells recovered from infected lungs of mice at 8 h and 24 h after infection. We compared the data with SAGE libraries from cells grown in iron-limiting medium and from cells harvested from the central nervous system of infected rabbits. The analysis revealed elevated expression of a set of functions at 8 h versus 24 h, including multiple transporters (sugar, iron, copper, amino acids and phosphate), as well as proteins involved in secretion, stress response, cellular respiration, protein and carbohydrate catabolism, and virulence. Interestingly, long SAGE analysis also revealed elevated expression of genes for lipid metabolism and for utilization of two-carbon compounds in cells grown *in vivo* compared with those grown in culture. In contrast, very few of transcripts were elevated at 24 h versus 8 h suggesting that dramatic transcriptional remodeling occurs during the early stages of infection. Real time PCR is being used to confirm the expression of selected transcripts. In addition, a subset of the genes identified by SAGE have been disrupted to**

determine whether the *in vivo* gene expression patterns indicate important functional roles during pulmonary infection. Taken together, these findings provide the first insight into the transcriptional response of *C. neoformans* during the pulmonary infection.

299. *Candida albicans CHT4* encodes a sporulation specific gene. Alexander Duenkler[1] and Jürgen Wendland[1,2]. [1] Department of Microbiology, Friedrich-Schiller-University, Jena and Junior Research Group: Fungal Pathogens, Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll Institute, Jena; Beutenbergstr 11a; D-07745, Jena, Germany. [2]Carlsberg Laboratory, Yeast Biology, Gamle Carlsberg Vej 10; DK-2500 Valby, Copenhagen, Denmark

The *C. albicans* genome encodes four chitinases, *CHT1, CHT2, CHT3,* and *CHT4*. In a recent analysis we have shown that Cht3 encodes the functional homolog of the *Saccharomyces cerevisiae* chitinase Cts1 that is involved in mother-daughter cell separation. All four chitinase encoding genes are non-essential. They belong to two groups in which CHT1, CHT2, and CHT3 are more similar to CTS1, while CHT4 is more similar to CTS2. ScCTS2 was described as a "sporulation specific gene".

However, published experimental evidence does not exist. The diploid cts2/cts2 strain sporulates well and the spores do not exhibit germination defects or increased sensitivity against zymolyase. In the filamentous fungus *Ashbya gossypii* a CTS2 homolog (ACL166w) was identified. The AgCts2 is 490aa in length and shows 42.3% overall identity to ScCts2 (511aa) and 33.2% identity to CaCht4 (388aa). The catalytic centers of the three proteins are highly conserved and shows clear differences to members of the Cts1-chitinase family. We deleted the AgCTS2 gene and carried out phenotypic characterizations. The Agcts2 mutants showed no growth retardation or morphogenetic defects. However, Agcts2 mutants revealed a spore shape defect indicating defects in the assembly of the spore wall. This defect did not lead to increased sensitivity against zymolyase or heat shock and did not affect the germination pattern of *A. gossypii* spores. Expression of AgCTS2 was analyzed using a lacZ reporter gene. AgCTS2 is expressed in the center of a mycelium that corresponds to the sporogenous mycelium.

300. Molecular Response to Putative Pheromone in *Histoplasma capsulatum*. Meggan Bubnick[1] and George Smulian[1,2]. [1]University of Cincinnati, [2]Cincinnati VA Medical Center.

***Histoplasma capsulatum (Hc)* is an ascomycetous human fungal pathogen found worldwide. *Hc* is a heterothallic organism with two described mating types, (+) and (-). There is a predominance of (-) mating type strains among clinical isolates; however, little is known about mating on a molecular level, and this must be resolved to address possible virulence differences between (+) and (-) mating type organisms. Predicted mating (MAT) locus idiomorphs, MAT1-1 and MAT1-2, pheromone receptors *STE2* and *STE3* and a predicted pheromone gene, *PPGA*, were identified in the *Hc* genome through homology and syntenic analysis. The predicted pheromone was synthesized, and used to stimulate two *Hc* clinical strains of opposite mating type, RE1 and VA1. Cultures were also stimulated with a and alpha pheromone extracts isolated from a co-culture of (-) and (+) mating type organisms. RNA was extracted, and quantitative RT-PCR was performed. Expression levels of pheromone receptor, *STE2* and the MAT locus transcription factors, *MAT1-1-1* and *MAT1-2-1*, were analyzed. In RE1, expression of *MAT1-1-1* and *STE2* increased with synthesized pheromone treatment, while in VA1, *STE2* and *MAT1-2-1* expression showed little change. *MAT1-1-1* expression levels in RE1 increased in response to alpha pheromone extract, while MAT1-2-1 expression levels in VA1 increased in response to a pheromone extract. The differential responses of *MAT1-1-1* and *MAT1-2-1* gene expression to pheromone stimulation confirm their role in regulation of mating type expression. Further studies are required to determine differential gene expression between organisms of opposite mating type and their role in virulence in *Hc*.**

301. Analysis of four putative beta–oxidation genes in *Aspergillus nidulans*. Kathrin Reiser, Meryl A. Davis and Michael J. Hynes. Department of Genetics, University of Melbourne

Filamentous fungi are able to use fatty acids as sole carbon sources via beta-oxidation. The enzymes required are present in both peroxisomes and mitochondria (Maggio-Hall and Keller 2004 Mol. Microbiol. 54:1173–1185). Two putative fatty acyl-CoA dehydrogenases, AcdA and AcdB, and two putative fatty acyl-CoA oxidases, AoxA and AoxB, were identified in the genome of *A. nidulans*. Because of their homology to Fox1p of *S. cerevisiae* and a peroxisomal dehydrogenase of *N. crassa*, they are thought to be involved in the first step of beta-oxidation. Homologues for each were found in other fungal species. Three of the proteins have a clear PTS1 (peroxisomal targeting sequence), while AcdA has a PTS1-like sequence. Hence, all four proteins are predicted to be peroxisomal. GFP fusion proteins have been constructed to investigate this hypothesis. In the 5' promoter region (1kb) of each gene, a 6bp sequence (CCGAGG/ CCTCGG) was found at least once. This sequence is predicted to be the core of a fatty acid depending regulation site (Hynes et al. 2006 Eukaryot. Cell. 5:794-805) suggesting positive regulation by fatty acids. Promoter-lacZ fusions have shown that at least two genes are induced by short- and long-chain fatty acids and induction is altered in the absence of the predicted regulatory proteins. Deletions

of all four genes do not give a severe fatty acid growth phenotype. Only the *aox*A-deletion phenotype is clearly visible. This implies a great redundancy amongst peroxisomal proteins involved in the first step of beta- oxidation.

302. Role of chromatin modifiers in secondary metabolite production in *Aspergillus nidulans.* Jin Woo Bok and Nancy P. Keller. Dept of Plant pathology, University of Wisconsin, Madison, U. S. A.

In eukaryotic cells, the DNA is packaged into chromatin by histone proteins. Post-transcriptional modifications of the histones result in epigenetic regulation of gene expression. Epigenetic processes are essential for development and differentiation in eukaryotic cells including fungi. Fungal secondary metabolites are also developmentally regulated. The connection between the epigenetic processes and secondary metabolite production is poorly understood. To unveil epigenetic regulation in secondary metabolite production, a well-known model fungus *Aspergillus nidulans* was used to introduce mutation in chromatin modifiers. Many chromatin modifiers are conserved from yeast to human including COMPASS (a histone 3 K4 methylase complex), Paf1 complex (a connecting complex between COMPASS and RNA polymerase II), Dot1 (a histone3 K79 methylase) and Lsd (a demethylase of histone3 K4). Here, we present results of secondary metabolite production modification in deletion mutants of those conserved chromatin modifiers. In addition, we examined a role of *laeA* (a global regulator of secondary metabolites) in these mutants.

303. Activation of the RNAi components as part of the double-stranded RNA induced host defense response in a filamentous fungus. Swati Choudhary[1,4], Heng-Chi Lee[1,4], Mekhala Maiti[1,4], Qun He[1,5], Ping Cheng[1,6], Qinghua Liu[2], and Yi Liu[1,3]. [1]Department of Physiology and [2]Department of Biochemistry University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390 USA [3]To whom correspondence should be addressed: E-mail: Yi.Liu@UTSouthwestern.edu [4]These authors contributed equally in this study. [5]Current address: State Key Laboratory for Agro-Biotechnology, College of Biological Sciences, Chinese Agricultural University, Beijing, 100094, China. [6]Current address: Merck Research Laboratories, 126 E. Lincoln Ave. Rahway, NJ 07065, USA

Double-stranded RNA (dsRNA) produced in eukaryotic cells can be recognized by the RNA interference (RNAi) pathway to result in posttranscriptional gene silencing. In addition, dsRNA can trigger interferon response as part of the immune response in vertebrates. In this study, we show that dsRNA, but not siRNA, can induce the expression of *qde-2* (an argonaute gene) and *dcl-2* (a dicer gene), two central RNAi components of the filamentous fungus *Neurospora crassa*. The induction of QDE-2 associates with enhanced efficiency of gene silencing, suggesting a regulatory mechanism that allows for the optimal function of the RNAi pathway. In addition, we show that DCLs regulate QDE-2 posttranscriptionally, suggesting a role for DCLs or siRNA in QDE-2 accumulation. Finally, genome-wide identification of dsRNA activated genes in *Neurospora* show that homologs of antiviral and interferon stimulated genes (ISGs), including Mx proteins, 6-16 family proteins, and a RNA helicase are highly induced by dsRNA. Together, our results suggest that the activation of the RNAi components is part of a broader ancient host defense response against viral and transposon infections.

304. QIP, a putative exonuclease, interacts with the *Neurospora* Argonaute protein and facilitates conversion of duplex siRNA into single strands. Mekhala Maiti, Heng-Chi Lee, and Yi Liu. Department of Physiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390 USA

Single-stranded siRNA guides the cleavage of homologous mRNA by Argonaute proteins, the catalytic core of the RNA-induced silencing complex (RISC), in the conserved RNA interference (RNAi) pathway. The separation of the siRNA duplex into single strands is essential for the activation of RISC. Previous biochemical studies have suggested that Argonaute proteins cleave and remove the passenger strand of siRNA duplex from RISC, but the *in vivo* importance of this process and the mechanism for the removal of the nicked passenger strand are not known. Here, we show that in the filamentous fungus *Neurospora*, the Argonaute homolog QDE-2 and its slicer function are required for the generation of single-stranded siRNA and gene silencing *in vivo*. Biochemical purification of QDE- 2 led to the identification of QIP, a QDE-2-interacting protein, with an exonuclease domain. The disruption of *qip* in *Neurospora* impaired gene silencing and siRNA accumulated, mostly in duplex form. Furthermore, our results suggest that QIP acts as an exonuclease that cleaves and removes the nicked passenger strand from siRNA duplex in a QDE-2 dependent manner. Together, these results suggest that both the cleavage and removal of the passenger strand from the siRNA duplex are important steps in RNAi pathways.

305. NmrA levels regulate the activity of AreA, the major nitrogen regulator, in *Aspergillus nidulans*. Koon Ho Wong, Richard B. Todd, Michael J. Hynes and Meryl A. Davis. Department of Genetics, The University of Melbourne, Parkville, 3010, Australia

The GATA transcription factor AreA is the major nitrogen regulatory protein in *Aspergillus nidulans*. AreA activates the expression of enzymes and permeases required for the breakdown of secondary nitrogen sources. The activator function of AreA is inhibited in the presence of primary nitrogen sources such as ammonium by interaction with the negatively

acting protein NmrA. We have investigated the effects of overexpression of AreA and NmrA using the highly inducible *xylP* promoter. Overexpression of AreA results in partial derepression of catabolic gene expression whereas overexpression of NmrA prevents AreA activity even in the absence of repressing metabolites. The levels of *areA* mRNA and AreA protein are low in the presence of ammonium and elevated during nitrogen limitation. We have shown that *nmrA* has the opposite pattern of expression with high levels of NmrA produced on ammonium. To localise sequences required for the regulated expression of *nmrA*, we have created promoter deletions in the *nmrA* gene and introduced these constructs into an *nmrA* deletion strain. Functional studies, together with RT-PCR and Western blot analyses, revealed that promoter sequences between -800 and -512 are required for full expression of NmrA and inhibition of AreA activity. Sequence analysis has revealed that several potential bZIP transcription factor binding sites are located within this region of the *nmrA* promoter.

306. Coordinated regulation of the melanin biosynthesis gene cluster in *Cochliobolus heterostrophus.* Sophie Lev[1§], Noa Eliahu[1], Aeid Igbaria[1], Mark S. Rose[2] and Benjamin A. Horwitz[1]. [1]Department of Biology, Technion - Israel Institute of Technology, Haifa, Israel, and [2]Syngenta Biotechnology Inc., North Carolina, USA. [§]email: sophia@tx.technion.ac.il

The maize pathogen *Cochliobolus heterostrophus* requires two MAP kinases, Chk1 and Mps1, to produce normal pigmentation. Young colonies of *mps1* and *chk1* deletion mutants have a white and autolytic appearance, which was partially rescued by a hyperosmotic environment. We isolated the transcription factor Cmr1, an ortholog of *Colletotrichum lagenarium* Cmr1 and *Magnaporthe grisea* Pig1, which regulates melanin biosynthesis in *C. heterostrophus*. Deletion of *CMR1* in *C. heterostrophus* resulted in mutants that lacked dark pigmentation, and acquired an orange-pink color. In *cmr1*-deletion strains the expression of putative scytalone dehydratase (*SCD1*) and hydroxynaphthalene reductase (*BRN1* and *BRN2*) genes involved in melanin biosynthesis was undetectable, whereas expression of *PKS18*, encoding a polyketide synthase, was only moderately reduced. In *chk1* and *mps1* mutants expression of *PKS18*, *SCD1*, *BRN1*, *BRN2*, and the transcription factor *CMR1* itself was very low in young colonies, slightly up-regulated in ageing colonies, and significantly induced in hyperosmotic conditions, as compared to invariable high expression in wild type. These findings indicate that two MAP kinases, Chk1 and Mps1, affect Cmr1 at the transcriptional level, and this influence is partially overridden in stress conditions including ageing culture and hyperosmotic environment. Surprisingly, we found that the *CMR1* gene was transcribed in both sense and antisense directions, apparently producing mRNA as well as a long noncoding RNA transcript. Expression of the antisense *CMR1* was also Chk1 and Mps1-dependent. Analysis of chromosomal location of the melanin biosynthesis genes in *C. heterostrophus* resulted in identification of a small gene cluster comprising *BRN1*, *CMR1* and *PKS18*. Since expression of all three genes depends on Chk1 and Mps1 MAPKs, we suggest their possible epigenetic regulation.

307. The NADH oxidase *nadA* and its involvement in oxidative stress in *Aspergillus flavus*. Carrie A. Smith[1], Massimo Reverberi[2], Niki Robertson[1], Gary A. Payne[1]. [1]North Carolina State University, Raleigh, NC, USA, 27606. [2]Università "La Sapienza," Rome, Italy 00165.

***nadA*, which encodes a predicted NADH oxidase, was identified as part of a sugar utilization cluster that lies adjacent to the aflatoxin biosynthetic cluster in several species of *Aspergillus*. NADH oxidases convert NADH to NAD+, which is a possible coenzyme needed for reactions in the aflatoxin biochemical pathway. In a microarray experiment comparing gene expression between a wild type strain of *A. parasiticus* and a deletion mutant for the pathway regulatory gene *aflR*, *nadA* expression was significantly decreased in the mutant background. Although *nadA* is transcriptionally controlled by AflR, aflatoxin levels were unaffected in *A. flavus nadA* deletion strains under several conditions. NADH oxidases can also be a source of reactive oxygen species formation. Previous reports have shown a relationship between oxidative stress and aflatoxin production in *Aspergillus sp*. The activity of several antioxidant enzymes were examined in *nadA* deletion strains and peak activity was delayed when compared to wild type. Lower levels of lipoperoxide accumulation were also seen in *nadA* deletion strains. These data suggest that *nadA* plays a role in the oxidative stress response in *A. flavus*. Investigations are underway to characterize additional phenotypes of *nadA* deletion mutants.**

308. Withdrawn

309. Transcriptional activators of *Aspergillus nidulans* sulfur metabolism. Sebastian Pilsyk, Jerzy Brzywczy and Andrzej Paszewski. Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawiñskiego 5A, 02-106 Warsaw, Poland e-mail: Seba@ibb.waw.pl

***Aspergillus nidulans metR* gene encodes a bZIP transcription factor specific for activation of several sulfur metabolism genes including these encoding the sulfate assimilation pathway enzymes. METR protein level and/or activity is negatively regulated by SCF ubiquitin ligase complex which consists of the proteins encoded by the *scon* (sulfur controller) genes. Both *metR* and *scon* genes constitute a regulatory system known as sulfur metabolite repression (SMR). Loss of function mutations in the *metR* gene cause methionine auxotrophy while mutations in the *scon* genes lead to a derepression of the**

sulfate assimilation pathway enzymes. All *metR* and *scon* mutants isolated so far were recessive. Recently, we have isolated three dominant suppressor mutants in a diploid strain homozygotic for *metB3* allele. Genetic and molecular analysis showed that Phe48 codon of the *metR* gene is affected in every of the dominant mutants obtained (*MetR20*, *21* and *22*). The orthologs of the *metR* gene were also found in other fungal species and Phe48 appears to be one of the most conserved amino acids of the N-terminal domain which suggests that this residue is important for METR protein function and/or stability. *MetR20* mutant has elevated acivity of the sulfur metabolism enzymes in comparison to the wild type. Suppressor *metR* mutants have also an increased level of sulfur compounds in mycelia which suggests that they are resistant to SMR. By searching of the *A. nidulans* genome sequence, we have found a paralog of the METR protein that encoded protein has similar basic region and leucine zipper sequences. The gene we named *metZ* is well conserved among other *Aspergilli* and it has apparently unusual large conserved intron as long as intron in the *metR* gene.

310. The role of MeaB, NMR and AreB as antagonists of AreA in *Fusarium fujikuroi.* Birgit Schönig, Anne Schmeinck, and Bettina Tudzynski. Universität Münster, Institut für Botanik, Schlossgarten 3, D- 48149 Münster, Germany

In *Fusarium fujikuroi*, the biosynthesis of gibberellins (GAs) and bikaverin, both nitrogen-free metabolites, is under control of AreA-mediated nitrogen metabolite repression. Glutamine and ammonium strictly repress the expression of AreA target genes. This strong repression can only partially be overruled by rapamycin-mediated inhibition of TOR suggesting that the activity of AreA is controlled by additional negative regulators. In contrast to *Aspergillus nidulans*, NMR was shown to play only a minor role as inhibitor of AreA activity. In addition to NMR, the bZIP transcription factor MeaB also negatively affects the expression of GA-and bikaverin biosynthesis genes as well as other AreA target genes. In the *meaB* deletion strain, these genes are up-regulated in a rapamycin-dependent manner. Interestingly, in double *areA/meaB* mutants, the de-repressing effect of the *meaB* deletion overrules the down-regulation of the AreA target genes in the single *areA* mutant. Target genes of AreA and MeaB were identified by an array-based approach. Furthermore, beside AreA, a second GATA-type transcription factor, AreB, is involved in nitrogen regulation. We present a putative model of nitrogen regulation in *F. fujikuroi* .

311. Identification of genes up- and down-regulated in the *nuc*-2A mutant strain of *Neurospora crassa.* Gras, DE[1], Silveira, HCS[1], Martinez-Rossi, NM[1] and Rossi, A[2]. [1]Departamento de Genética, FMRP-USP [2]Departamento de Bioquímica e Imunologia, FMRP-USP, Ribeirão Preto, Brazil.

Microorganisms have evolved complex signal transduction networks that enable them to make optimal use of the nutrient sources available. Inorganic phosphate (Pi), an essential nutrient for all organisms, is required for the biosynthesis of nucleic acids, phospholipids and cellular metabolites, as well as for the energy transduction and metabolic signaling responses. The phosphorus acquisition system in *Neurospora crassa* includes four regulatory genes: *nuc*- 2$^+$, *preg*$^+$, *pgov*$^+$, and *nuc*-1$^+$. Under limiting Pi, NUC-2 inhibits the functioning of the complex PREG-PGOV through the interaction of ankyrin-like repeats, allowing the activation of the wide domain transcription regulator NUC-1. In an attempt to identify genes involved in metabolic responses to exogenous Pi sensing, we employed suppression subtractive hybridizations (SSH) between RNA isolated from the wild type (*St*.L.74A) and *nuc*-2A (FGSC#1996) grown under Pi starvation, pH 5.4. Following SSH, expression of clones was examined using dot-blot macro-arrays. Of the 900 clones arrayed from the SSH cDNA library, approximately 21% were differentially regulated. A total of 66 differentially up-regulated and 124 down-regulated clones were identified and sequenced. Genes encoding proteins involved in signal transduction and regulation were identified among the up-regulated sequences, including translation initiation factor eIF3, ubiquitin and kinases. Among the genes found to be down regulated in *nuc*-2A, six were involved in gluconeogenesis and oxidative phosphorylation. Virtual and Northern blot analyses of randomly selected genes confirmed the differential gene expression result. Thus, the identification of differentially-regulated genes in *nuc*-2A is key to the understanding of molecular events involved in phosphorus sensing.

312. Involvement of Ace2 (Activator of Cellulases 2) in the architecture of the *xyn2* transcriptosome of *Hypocrea jecorina.* Astrid R. Stricker*, Peter Trefflinger, Nina Aro, Merja Penttilä, Robert L. Mach *e-mail:stricker@mail.zserv.tuwien.ac.at

Ace2 (Activator of Cellulases 2)-encoding gene was deleted and retransformed in the *H. jecorina* QM9414 genome. Comparison of xylanase formation and *xyn2* transcription of the corresponding strains after cultivation in a batch fermentation or replacement to inducing compounds (xylan, xylobiose) revealed the deletion of *ace2* to be responsible for a faster initial inducibility, but final levels of *xyn2* transcript as well as xylanase activity formation of the parental strain cannot be reached. Ace2 is not only involved in induction mechanisms but it is also responsible for basal level of *xyn2* transcription. Interestingly, Ace2 does not mediate sophorose-specific induction although it plays a role in protein/DNA complex formation under sophorose-inducing conditions. Furthermore, in the present study, identification of a palindrome in the *xyn2* promoter consisting of a GGGTAA- and a CCAGCC-element (closely resembling the binding site

of Xyr1) is given. Both Xyr1 as well as Ace2 are able to bind the complete motif, the latter also only to one part of it. Phosphorylation as well as dimerization are shown to be a prerequisite for binding of Ace2 to the *xyn2* promoter. Finally impact of Ace2 on *xyr1* transcription could be demonstrated under inducing conditions. Summarizing, Ace2 can be considered as a factor modulating the overall activation influence of Xyr1 concerning *xyn2* expression in the style of Ace1/Xyr1 interaction. This may involve an interplay of both factors on protein/DNA basis as well as direct modulation of *xyr1* transcript formation.

313. Differential regulation of xylanase gene expression in *Hypocrea jecorina* is due to the interplay of the general activator Xyr1 and the fine tuning transcription factors Ace1 and Ace2. Robert L. Mach*, Astrid R. Stricker, Roman Rauscher, Elisabeth Würleitner, Peter Trefflinger * rmach@mail.zserv.tuwien.ac.at

Enzymes of *H. jecorina* (anamorph *Trichoderma reesei*) capable of degrading the xylan backbone (encoded by the genes *xyn1* and *xyn2*) have received strong attention because of their application in feed and paper industry. Whereas in *Aspergillus* the xylanolytic system is strictly co-regulated via the inducer xylose enzymes participating in the respective *T. reesei* hydrolytic complexes are not. In *Trichoderma* these more complex induction mechanisms are conferred by modulation of the general regulator of hydrolase formation Xyr1 in its mode of action by additional narrow domain transcription factors such as Ace1 and Ace2. In the case of Ace1/Xyr1-driven *xyn1* expression we will shown that Ace1 is a competitor of Xyr1 for only one of the two binding elements. Ace2 is involved in several aspects of *xyn2* expression if the inducer is xylobiose or the fungus is cultivated on xylan. Interestingly, Ace2 is not only responsible for the basal transcription of *xyn2* but also is important for particular steps of induction. Obtained data indicate that Ace2 acts in a dual role, on the one hand as an antagonist of early induction and on the other hand as an enhancer of a continuous extension of expression of *xyn2*. Our study revealed a spectrum of different mechanisms modifying the Xyr1-dependent transcriptosomes of *xyn1* and *xyn2* respectively, including phosphorylation, competition, homo- and heterodimerisation and recruiting of additional regulatory proteins.

314. Expression and transposition of DNA transposon *Crawler* in *Aspergillus oryzae*. Hironobu Ogasawara[1], Hiroshi Obata[2], Yoji Hata[2], Saori Takahashi[1], and Katsuya Gomi[3]. [1]Akita Research Institute for Food and Brewing, Akita, Japan. [2]Research Institute, Gekkeikan Sake Co. Ltd., Kyoto, Japan. [3]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan.

An active DNA transposon *Crawler* has been isolated and characterized from the industrially important fungus *Aspergillus oryzae*. The transposition events of *Crawler* were induced by various stress treatments such as $CuSO_4$ or heat shock. The existence of two or more transcripts in different size of *Crawler* was shown under standard culture conditions. In the present study, we analyzed the transcripts of different size by 3'-RACE analysis. Moreover, relationship between the transposition activity and the proportions of *Crawler* mRNA molecules was also studied to clarify the control mechanism for transcription of exogenous gene *Crawler*. The smaller transcribed fragments were resulted from premature polyadenylation and in some cases erroneous intron splicing within the transposase. The erroneous splicing tends to be inhibited by stress treatment of $CuSO_4$, which stimulated the transposition events in conidia allowing the full-length and active transposase to be produced. These results indicate that *A. oryzae* has a defense system against the exogenous active genes like transposons by mRNA quality control system such as undesirable splicing or polyadenylation resulted in nonstop mRNA decay.

315. Transcription independent chromatin remodelling in *Aspergillus nidulans*. Harald Berger, Asjad Basheer, Sandra Böck and Joseph Strauss. Fungal Genomics Unit, Austrian Research Centers and BOKU Vienna, Austria.

In *Aspergillus nidulans* the induction of nitrate assimilating genes (*niiA, niaD*) is associated with chromatin remodelling processes which depend on the function of a GATA-factor (AreA) and a synergistically acting activator (NirA). Functional dissection of the process revealed that, in addition to nucleosome loss-of-positioning, AreA functions as independent transcriptional activator and also interacts with NirA to stimulate its DNA-binding capacity. Here, we analyzed NirA and AreA-dependent histone acetylation and nucleosomal DNA accessibility at different time points of nitrogen starvation and nitrate induction in the promoter region of *niiA* and *niaD*. We find that accumulation of AreA under starvation conditions leads to increased histone H3 acetylation and loss of nucleosome positioning and that this processes are abolished in an *areA*$^-$ strain. High H3 acetylation levels are associated with loss of nucleosome positioning but not with early transcriptional activation. In contrast, transcriptional activation which is strictly dependent on NirA, does not lead to H3 hyperacetylation and only partial nucleosomal loss of positioning is observed under these conditions. Our results indicate that transcriptional activation is not necessarily associated with a drastic chromatin rearrangement in the promoter but AreA-mediated hyperacetylation of H3 is required for maintaining an open chromatin structure facilitating continued transcription from this promoter.

316. Identification of genes up regulated in the *pal*A1 mutant strain of *Aspergillus nidulans*. Emiliana M. Silva[1], Janaina S. Freitas[1], Diana Ester Gras[2], Juliana Leal[2], Fabio M. Squina[1], Henrique S.C. Silveira[2], Nilce M. Martinez Rossi[2], Antonio Rossi Filho[1]. [1]Departamento de Bioquímica e Imunologia, FMRP-USP, Brazil [2]Departamento de Genética, FMRP-USP, Brazil e-mail: mimandarano2002@yahoo.com.br

The conserved PacC signal transduction pathway mediates many metabolic events involved in ambient pH sensing in *Aspergillus nidulans*, and it is widely accepted that it governs the response to neutral-to-alkaline pH. The *pac*C gene codes for a Zn-finger transcription factor, and the six *pal* genes (*pal*A, B, C, F, H, and I) are putative members of a signaling cascade, which promotes the proteolytic activation of PacC in alkaline environment. The PalA protein interacts with the YPXL/I motifs in PacC, what is required for the action of PalB, a calpain-like protease. Thus, whatever the growth pH is, *pal*A1 mutation should cause an acidity-mimicking phenotype. This model implies that loss-of-function mutation in any of the six *pal* genes shall lead to a wild-type acidic growth phenotype regardless on the ambient pH, what was not observed in the *pal*A1 mutant strain. Employing suppression subtractive hybridization, we identified genes up-regulated in the *pal*A1 mutant strain of *A. nidulans* grown in low-Pi medium, pH 5.0, whose expression should occur only at alkaline pH, as is the case for the expression of the *phospholipase* C gene. This result shows a derangement in the gene expression profile at pH 5.0, indicating that the *pal*A gene is functional at acidic pH and, therefore, the *pal*A1 mutation did not cause an acidic-mimicking phenotype. Financial support: FAPESP, CAPES, CNPq and FAEPA.

317. Regulated transcript stability in *Aspergillus nidulans*. Mark X Caddick, Sunthorn Chooluck, Meriel Jones and Igor Morozov. School of Biological Sciences, University of Liverpool, UK. caddick@liv.ac.uk

The level of any transcript is defined by the combined rates of transcription and degradation, both of which are regulated. Nitrogen metabolism in *Aspergillus nidulans* involves coordinated expression of hundreds of genes, many dependent on the transcription factor AreA, which monitors the nitrogen state of the cell. AreA activity is in part modulated by differential degradation of its transcript in response to intracellular glutamine. We have also determined that glutamine triggers synchronised degradation of a large subset of transcripts involved in nitrogen metabolism. Significantly, we show that two of these transcripts, *niaD* and *niiA*, are also stabilised by intracellular nitrate, directly reinforcing their transcriptional regulation. Gln-mediated degradation and the nitrate-dependent stabilisation of the niaD transcript are effected at the level of poly(A) deadenylation and are dependent on the 3' UTR. To investigate the mechanisms involved in regulated deadenylation we have systematically disrupted a series of genes identified by homology. These include genes encoding three putative deadenylases and five PUF proteins. We have identified and characterised additional components by MS/MS sequencing of proteins specifically associated with the areA 3' UTR. We will present our current model for the regulated degradation of transcripts in response to nitrogen availability.

318. Identification of genes down regulated in the *preg*^{c} mutant strain of *N. crassa*. Fabio M. Squina[1], Juliana Leal[2], Nilce M. Martinez-Rossi[2] and Antonio Rossi[1]. [1]Departamento de Bioquímica e Imunologia and [2]Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil.

***N. crassa* synthesizes nucleases, phosphatases, and Pi transporters in response to signals of Pi starvation, and their regulation includes at least four genes involved in a hierarchical relationship: *nuc-2*, *preg*, *pgov* and *nuc- 1*. The PREG/PGOV proteins form a cyclin-CDK complex that regulates negatively the translocation of the transcription factor NUC-1 into the nucleus. Nevertheless, cyclins seem to have important participation in other metabolic pathways involved in cell cycle functions by adding specificity to the cyclin-CDK complex. Thus, loss-of- function mutations in the *preg* gene may alter the expression of other genes rather than those for the Pi- repressible phosphatases. In the present study we showed, by employing differential display reverse transcription polymerase chain reaction, that the expression of the genes for iso-orotate decarboxylase (IDCase) and ubiquinone metiltransferase is reduced in the *preg*^{c} mutant strain grown under Pi shortage. The IDCase is a step of the thymidine salvage pathway, which reuses pyrimidines to form nucleotides more advantageously than *de novo* biosynthesis, and also contributes to maintain the balance in the nucleotide metabolism. It is expected that the pathways for the biosynthesis, catabolism and interconversion of critically important molecules, the nucleotides for instance, are highly integrated and highly regulated. Thus, disruption of the UMP/UDP pool balance by lowering the expression of an enzyme of the thymidine salvage pathway would have a variety of physiological effects in the cell's life, including both growth and conidiation of the mutant strain. Financial support: FAPESP, CNPq, CAPES and FAEPA**

319. Regulation by Blue Light and Heat Shock of Gene Transcription in *Phycomyces blakesleeanus*: Proteins Required for Photoinduction and Identification of a Novel Mechanism for Adaptation to Light. Julio L. Rodriguez-Romero and Luis M. Corrochano. Departamento de Genetica, Universidad de Sevilla, Spain

The gene *hspA* for the heat-shock protein HSP100 is induced by blue light and heat shock in the zygomycete fungus *Phycomyces*. HSP100 proteins are ATPases involved in the tolerance to high temperatures, proteolysis, and protein

disaggregation. We have identified the gene products required for *hspA* photoactivation and found that many of them are also required for mycelial photoresponses, a suggestion for a common signal transduction pathway. The photoactivation of *hspA* requires the protein MADA, with a Zn finger and a flavin-binding site, the *madB* gene product, which is also required for all the photoresponses, and a regular supply of beta-carotene. None of the *madA* alleles that result in mutations in the flavin-binding site or that lacks the Zn finger allow *hspA* photoactivation suggesting that MADA is a photoreceptor and a transcription factor responsible for gene photoactivation probably through the formation of a complex with a protein similar to *Neurospora* WC-2. The activation of *hspA* after blue light-exposure or a heat shock is transient. The adaptation of *hspA* photoactivation is not the result of photoreceptor desensitization, but the result of a novel mechanism causing a light- dependent blockage of gene transcription. The light-dependent blockage of gene transcription does not prevent the activation of hspA by a heat shock and may be caused by the depletion of the MADA transcription factor from the hspA promoter. Our results have allowed us to propose a model for the regulation of *hspA* transcription by blue light and heat shock.

320. Mutations in a Gene Repressor or a Glucose Transporter Result in Sustained Gene Photoactivation in Neurospora. Maria Olmedo, Laura Navarro-Sampedro and Luis M. Corrochano. Departamento de Genetica, Universidad de Sevilla, Spain mariaolmedo@us.es

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. The *rco* mutants were isolated by the abundant expression of *con-10* in vegetative mycelia. The gene *rco-1*, encoding a putative gene repressor, and the gene *rco-3*, encoding a putative glucose sensor, are required for the repression of *con- 10* in vegetative mycelia. We have observed that *rco-1* and *rco-3* mutants have an enhanced and sustained photoactivation of *con-10* and *con-6*, a phenotype they share with *vivid* mutants. The abundant photactivation of *con-10* and *con-6* in *rco* and *vivid* strains is best observed after five hours of light. The *rco* and *vivid* mutations do not alter the stability of the *con-10* and *con-6* mRNAs, suggesting that the sustained photoactivation is due to a high transcriptional rate that is not subjected to adaptation to light. The threshold of *con-10* and *con-6* photoactivation is significantly lowered in the *rco-1* mutant, but remains unchanged in the *rco-3* and *vivid* mutants. The photophosphorylation of WC-1 in the *rco-1*, and *rco-3* strains seem to be similar to that of the wild type in contrast to the mutation in *vivid* that results in a sustained WC-1 photophosphorylation. The *rco-1* mutant contains a point mutation at nt 1986 resulting in a change of Ser by Phe in the conserved WD domain. We propose that VVD, RCO-1, and RCO-3 seem to participate directly or indirectly in the mechanism of adaptation to light.

321. A genome approach to understanding nonself recognition, heterokaryon incompatibility and programmed cell death. Elizabeth A. Hutchison, Karine Dementhon, and N. Louise Glass. Plant and Microbial Biology department, UC Berkeley, California, 94720

***Neurospora crassa* can undergo hyphal fusion during vegetative growth to form a heterokaryon. Heterokaryons are only viable between genetically identical individuals; differences at any of 11 heterokaryon (*het*) loci result in growth inhibition and death. *het-c/pin-c* mediated nonself recognition is a model to study heterokaryon incompatibility (HI) and the cell death response. The *het-c* locus encodes a plasma membrane protein while *pin-c* encodes a HET domain protein. The HET domain is a conserved protein motif that is present in almost all characterized HI interactions in *N. crassa* and *Podospora anserina*. Mutations at the *vib-1* locus, which encodes a putative transcription factor, suppress HI. Based on Q-RT-PCR data, *vib-1* was required for the expression of 3 of the 55 HET domain genes, including *pin-c, tol* and *het-6*. Comparative genome hybridization was used to identify additional polymorphic HET domain genes; 6 of 8 additional HET domain genes showed significantly decreased expression in the absence of *vib-1*. *vib-1* is also required for protease production during nitrogen and carbon starvation. Mutants in two *vib-1* paralogs, NCU04729 and NCU09915, will be examined for protease production deficiencies during nutrient starvation. Finally, the effects of both the *vib-1* regulated HET domain genes and the *vib-1* paralogs on heterokaryon incompatibility will be investigated.**

322. *mat1-2* gene and cryptic fertility in *Aspergillus fumigatus.* Wioletta Pyrzak, Bruce L. Miller, Karen Miller. Department of Microbiology Molecular Biology and Biochemistry, University of Idaho, Moscow, ID, U.S.A

The lack of sexual cycle in *Aspergillus fumigatus (Af)* is a major limitation in the study of its pathogenesis. Recent genomic studies revealed evidence for potential sexuality in *A. fumigatus.* The *mat1-2* gene encodes a homologue of the MatA HMG-box mating transcriptional factor that regulates sexual development in fertile *Aspergillus nidulans (An).* A non functional *mat1-2* gene or HMG-box protein could be one reason for loss of sexuality in *A. fumigatus.* Gene complementation and ORF swap experiments were performed to determine a potential functionality of *Af mat1-2* gene in the control of sexual cycle. Results showed that *Af mat1-2* did not complement an *A. nidulans matA* deletion strain. No sexual cycle was observed. An ORF swap gene replacement demonstrated that Mat1-2 protein functionally replaced *A. nidulans* MatA protein during sexual development. Sexual development was delayed 1-2 days and cleistothecia were 20 x larger relative to wild type. Normal number and viability of ascospores per cleistothecium were observed. Incomplete asci

formation and ascospores maturation were observed after 12 days of development. This data suggest that *A. fumigatus* Mat1-2 protein is functional and can drive sexual reproduction in *A. nidulans.* Results presented in this study provide evidence for potential cryptic sexual activity in *Aspergillus fumigatus.*

323. Identification of carbonic anhydrases in *Aspergillus fumigatus* and *A. nidulans.* Kap-Hoon Han[1], Hyoun-Young Kim[1], Jong Hwa Kim[1], and Yong-Sun Bahn[2]. [1]Dept. of Pharmaceutical Engineering, Woosuk University, Wanju, 565-701, Korea. [2]Dept. of Bioinformatics & Life Science, Soongsil University, Seoul, 156-743, Korea.

Like vertebrates, carbon dioxide (CO2) plays an important role in respiration of various microorganisms including fungi. Furthermore, differential CO2 concentration can act as a signaling cue. Since mammals maintain high CO2 concentration (~5%) compared to natural environment (~0.036%), animal fungal pathogens must adapt to high CO2 levels for their infection. Although CO2 sensing and metabolism in filamentous fungi have been poorly understood, it has been well known that the reversible interconversion between gaseous CO2 and bicarbonate ions (HCO3-) by carbonic anhydrase (CA) is key for maintaining cellular homeostasis. Since *Aspergillus fumigatus* is a major fungal pathogen causing invasive aspergillosis in immunocompromised patients, adaptation of different CO2 concentration, probably mediated by CAs, could be important. Genome sequence of *A. fumigatus* and *A. nidulans* allowed us to identify three conserved CAs in each organism that are designated as *cafA* (CA in *A. fumigatus*), *cafB* and *cafC*, and *canA* (CA in *A. nidulans*), *canB* and *canC*, respectively. Interestingly, plate sealing caused upregulation of *cafA* and *canA* expression, suggesting that the limited aeration leads to the shortage of CO2 which promotes upregulation of the CA expression.

324. Cwc1 and Cwc2 are central regulators of blue light photoresponses in *Cryptococcus neoformans*. Yu-Ling Yeh[1], Yu-Sheng Lin, Bei-Jia Su, and Wei-Chiang Shen[2]. Plant Pathology & Microbiology, National Taiwan Univ. [1] r94633012@ntu.edu.tw ; [2] wcshen@ntu.edu.tw

Light is one of the most important environmental factors regulating developmental and physiological processes in filamentous fungi. Our prior studies have demonstrated that blue light negatively regulates the sexual filamentation via the Cwc1 and Cwc2 proteins in *Cryptococcus neoformans*, a heterothallic basidiomycetous yeast. To reveal their roles in the regulation of light responses, we generated both *CWC2* overexpression strain under the *cwc1* mutant background and *CWC1* overexpression strain under the *cwc2* mutant background. The mating phenotypes of these strains were similar to those of the *cwc1* and *cwc2* mutant strains. These results suggested that Cwc1 and Cwc2 proteins function interdependently to regulate the light response. Furthermore, to reveal the importance of putative domains identified in the Cwc1 and Cwc2 proteins, we generated partially deleted versions of both proteins under the constitutively expressed *GPD1* promoter. Our results confirmed that LOV domain and PAS domains are essential for the function of Cwc1 protein, and PAS domain and zinc finger DNA-binding domain are also crucial for the Cwc2 protein. Additionally, to dissect the photoresponses mediated by Cwc proteins, we used *Agrobacterium* T-DNA insertional mutagenesis as an approach. A mutant suppressed the *CWC1* overexpression phenotype under light condition was identified, and T-DNA was found to integrate at the promoter region of the *CWC2* gene. Our results demonstrated that Cwc1 and Cwc2 proteins are two central regulators which form complex to regulate blue light photoresponses in *C. neoformans*.

325. Isolation and functional analysis of the *veA* interacting *vip* genes of *Aspergillus nidulans.* Hyoun-Young Kim[1], Hee-Seo Kim[2], Miae Oh[2], Kap-Hoon Han[1], Keon-Sang Chae[2], Jong Hwa Kim[1]. [1]Dept. of Pharmaceutical Engineering, Woosuk University, Jeonbuk 565-701, [2]Div. of Life Science, Chonbuk Nat'l University, Jeonbuk, 561-756, Korea.

The *vipA, B,* and *C* (VeA-interacting protein) genes were isolated by using yeast two hybrid analysis. The *vipA* transcript size was 1.3 kb and had an ORF encoding a 334 amino acid polypeptide. Putative *vipA* protein contains FAR1 domain related to plant-specific phytochrome signaling, suggesting that the VipA may act as a component of far-red light signaling with VeA in *A. nidulans*. However, *vipA*-null mutants and over- expressors did not show any remarkable phenotypic difference from a wild type. The *vipB* and *vipC* genes were also isolated. The *vipB* gene had an ORF encoding 332 amino acid polypeptide. The amino acid sequence of VipB showed a 38% identity to that of a methyltransferase. The *vipB* gene dose not seem to be involved in both of sexual development and asexual development, since the *vipB* deletion and overexpressoion mutants did not show any phenotypic difference from their control. The *vipC* negatively regulates sexual development in the presence of light, but not in dark condition. For obtaining further information of the genes, microarray analysis was carried out with RNA samples isolated from mycelia at the vegetative stage of three *vip*-deletion mutants, and their results were compared.

326. Gene expression analysis under membrane-transfer culture. Motoaki Sano[1], Akiko Kobayashi[1], Hiromoto Hisada[2], Yoji Hata[2], Masayuki Machida[3], Shinichi Ohashi[1]. [1]Kanazawa Institute of Technology (KIT), Hakusan, Japan [2]Res. Inst., Gekkeikan, Kyoto, Japan [3]Natl. Inst. of Advanced Ind. Sci. and Technol. (AIST), Tsukuba, Japan

Aspergillus oryzae **is one of the most useful microorganisms in many fields of biotechnology; has been used to produce fermented foods such as soy sauce, miso, and sake. In *A. oryzae*, the gene expression patterns change depending on the culture conditions, and a lot of important enzymes are expressed in solid-culture (SC), but not in submerged culture. However, SC has several disadvantages, such as mass transfer limitation inside the solid substrate and difficulty of control of substrate concentration as well as pH of the medium during cultivation. To overcome these disadvantages of SC, membrane-transfer culture (MTC) was developed. In MTC, *A. oryzae* are grown onto the surface of the nitrocellulose membrane. The gene expression patterns in MTC were analyzed by using DNA microarray consisting of 11,000 oligonucleotides. The DNA microarray analysis showed that there is global resemblance between MTC and SC in the gene expression. As for a part of SC-strong expression gene, it was confirmed to strongly express in MTC. Some genes which as specifically expressed in MTC were identified, and the promoter analysis of these genes were done.**

327. The role of XprG in the response to nutrient limitation in *A. nidulans.* Margaret E. Katz[1], Katharyn S. Sue[1], Richard B. Todd[2] and Brian F. Cheetham[1]. [1]University of New England, Armidale, NSW, Australia and [2]University of Melbourne, VIC, Australia. mkatz@une.edu.au.

We have identified three genes involved in the regulation of extracellular proteases in response to carbon, nitrogen and sulfur limitation. Two of the genes encode atypical hexokinases, HxkC and HxkD (formerly XprF), and the third gene product, XprG, belongs to a newly defined class of DNA-binding proteins. Vib-1, a regulator of genes required for programmed cell death in *N. crassa* is a homolog of XprG. Genetic evidence indicates that HxkC and HxkD are negative regulators of XprG. We have recently found that unlike HxkD, which is a nuclear protein, HxkC is associated with mitochondria. Mitochondrial hexokinases have been shown to block apoptosis in mammals and programmed cell death in plants. HxkC may play a similar role in filamentous fungi. *xprG*$^{-}$ mutants are protease- deficient and have pale conidia suggesting the XprG is involved in regulating extracellular protease and conidial pigment production. To investigate whether XprG is required for expression of other genes in response to carbon starvation, we used microarrays provided by the PFGRC. These experiments indicate that expression of many genes is greatly reduced in an *xprG*$^{-}$ null mutant. Many of the genes with altered expression (*e.g. brlA*, genes encoding sugar transporters and genes in the sterigmatocystin biosynthetic pathway) are known to be regulated in response to carbon starvation. These results indicate that XprG may play a major role in the response to carbon limitation. Nuclear accumulation of the nitrogen regulator, AreA, is abolished in an *xprG*$^{-}$ mutant.

328. Transcriptional Control and Protein Specialization have Roles in the Functional Diversification of Two Dicer-like Proteins in *Magnaporthe oryzae.* Naoki Kadotani, Hitoshi Nakayashiki. Lab of Plant Pathology Kobe University, Kobe Japan

Two Dicer-like genes, *Mdl-1* and *-2*, were identified in the genome of the rice blast fungus *Magnaporthe oryzae*. Molecular phylogenetic analyses indicated that the paralogs of the *Mdl-1* and *-2* genes diversified prior to the divergence of the major fungal lineages. Analyses of *Mdl-1* and *Mdl-2* knockout (KO) mutants revealed that *Mdl-2* was solely responsible for the siRNA-mediated gene silencing pathway in the vegetative phase of the fungus where *Mdl-2* mRNA was expressed 15 times more highly than was Mdl-1 mRNA. However, overexpression of the full-length *Mdl-1* cDNA compensated, albeit partially, for the RNA silencing- deficient phenotype of the *Mdl2* KO mutant. The levels of siRNA accumulations in the transformants with *Mdl-1* cDNA were significantly lower than in those with *Mdl-2* cDNA, even when they were overexpressed at similar levels. Our results indicated that the functional diversification of Mdl-1 and Mdl-2 proteins in RNA-mediated gene silencing pathways was likely to have come about from both transcriptional control and protein specialization.

329. MINUTE, the unusual a-type pheromone of *Hypocrea jecorina* (*Trichoderma reesei*) mediates light regulation of cellulase gene expression. M. Schmoll, M. Dorrer, and C. P. Kubicek. Institute of Chemical Engineering, TU Wien, Getreidemarkt 9-1665, A-1060 Vienna, Austria

Light has recently been shown to enhance the induced expression of cellulases in *H. jecorina.* In a cDNA subtraction assay targeted at genes specific for cellulase induction, we isolated minute (min1) encoding a small (5.1 kDa), alkaline (pI 9.6) α/β-protein, which is composed of three ß-sheets containing the motif (L/I)GC(S/T)VM and two helices. Thus, together with its CAAX-domain at the C-terminus MIN1 shares characteristics of both a- and alpha-type peptide pheromone precursors. It inhibits growth in *H. jecorina* and enhances perithecia formation *H. jecorina* CBS999.97. Transcription of min1 is up-regulated upon growth on cellulose in the wild-type strain QM9414 in light. Deletion of min1 results in loss of light-dependent modulation of cellulase gene expression on cellulose. Minute shows light dependent transcription, which is strongly enhanced in a strain in which the *H. jecorina* orthologue of the *N. crassa* light modulator VIVID – ENVOY – is non-functional. EMSAs using the cbh2 promoter and cell-free extracts from mycelia grown in light or darkness revealed DNA-protein complexes from the min1-deletion strain do not show the difference between cultivation in light or darkness as found in the wild-type. Thus, the unusual peptide pheromone precursor MINUTE is an important factor in light

regulation of cellulase gene expression and after ENVOY it represents the second component of the respective signal transduction pathway in *H. jecorina.*

330. Transcriptional profiling of *Candida albicans* late biofilm developmental stage associated with community mobilization. Adnane Sellam[1], Peter Suci[2], Thamir Al Niemi[2], Jean-Sébastein Deneault[1] and André Nantel[1]. [1]Biotechnology Research Institute, National Research Council of Canada, Montréal, Québec, Canada [2]Center for Biofilm Engineering, Montana State University-Bozeman, Montana

***Candida albicans* is a major cause of morbidity and mortality in bloodstream infections and is the most common fungal pathogen isolated from immuno-compromised patients. *C. albicans* is also implicated in many biomaterial- related infections which are typically associated with biofilm formation. One of the main consequences of the biofilm mode of growth is the increased resistance to antimicrobial therapy, which is the main reason why biofilm-associated infections are frequently refractory to conventional antibiotic therapy. Yeast cells of *C. albicans* have an important role in initiation of biofilm formation and its maintenance by anchoring its basal layer to the substratum. Recent studies suggest that yeast cells have also a key role in biofilm dispersal. These cells may detach from mature biofilm and colonize a new substrate area that is not nutrient deprived, where a new biofilm can develop unhindered. Until now nothing is known concerning the genetic program controlling biofilm cell mobilization. To characterize the detachment stage of yeast cells, we recovered mature biofilm and detached cells from a special flow- bioreactor and performed a detailed transcriptional analysis using a new generation of long oligonucleotide microarray. Four to six biological replicates from these populations were compared to each other and against yeast cells from regular batch cultures. For instance, we identified 249 significantly-modulated transcripts that best distinguish mature biofilm from yeast cells grown in batch culture. In this comparison, we did not identify significant changes in sulfur and amino acid metabolism as previously reported in other bioreactors (García-Sánchez et al. 2004) but we did observe a very significant correlation with the changes in transcriptional profiles that are associated with repression of the Polo-like kinase Cdc5p (Bachewich et al., 2005) suggesting that cell detachment is linked to mechanism regulating cell cycle in *C. albicans*. Additional results of these studies will be shown, discussed and progresses on functional characterization of candidate genes will also be presented.**

331. Withdrawn

332. Effect of Copper on the Expression of the Mco1 Gene from *Phanerochaete chrysosporium* and its Connection with the ACE1 Transcription Factor. Canessa, Paulo; Alvarez, Jose Miguel; Rivas, Alexis; Bull, Paulina & Vicuna, Rafael. Universidad Catolica de Chile, Fac. de Ciencias Biologicas, Lab. de Bioquimica, Alameda 340, Santiago, Chile. pcanessa@bio.puc.cl

We have previously identified a gene encoding ACE1, the first transcription factor described and functionally characterized in the ligninolytic fungus *Phanerochaete chrysosporium* (Pc-ACE1). To date, the possible target gene(s) of ACE1 remain elusive. Since *mco1* possesses at least two ACE elements in its promoter, we decided to analyze the effect of copper on the expression of this gene. Real-time RT-PCR experiments showed a 4 to 5-fold increase in the *mco1* mRNA levels upon copper supplementation of cultures. To further characterize Pc-ACE1, the protein was synthesized *in vitro* using a commercially available transcription-translation system. By means of electromobility-shift assays (EMSA) and using a DNA probe containing a previously described ACE element from yeast, we showed specific binding of Pc-ACE1 to the probe. In addition, EMSA also revealed that Pc-ACE1 binds specifically to a probe containing one of the putative ACE elements found in the *mco1* promoter. These results do not only show that Pc-ACE1 indeed recognizes a canonical ACE element, but also support the hypothesis that *mco1* is regulated by the Pc-ACE1 transcription factor. We are now identifying the key nucleotide residues involved in this protein-DNA interaction by site-directed mutagenesis. Financed by grant FONDECYT 1030495 & Millennium Institute (MIFAB)

333. A novel mechanism of regulation of iron-dependent pathways in eukaryotes is mediated by HapX interacting with the CCAAT-binding complex. Martin Eisendle[1], Birgit Seeber[1], Ernst R. Werner[2], and Hubertus Haas[1] [1]Division of Molecular Biology/Biocenter, and [2]Division of Biological Chemistry/Biocenter, Innsbruck Medical University, Fritz-Pregl-Str. 3, A-6020 Innsbruck, Austria; martin.eisendle@i-med.ac.at

Subtle control systems are required to maintain iron homeostasis as this metal is both essential and potentially toxic. In *Aspergillus nidulans* iron starvation induces iron acquisition but represses iron dependent pathways. Here we demonstrate that HapX mediates the transcriptional repression of iron-dependent pathways, e.g. heme biosynthesis, via physical interaction with the CCAAT-binding complex (CBC). The transcriptional regulation of iron acquisition, e.g. the siderophore system, is mediated by the GATA-type transcription factor SreA. Mutual transcriptional control of HapX and SreA and synthetic lethality of deletion of both regulators suggests a tight interplay of these two iron homeostatic control systems. Growth phenotypes indicate that the CBC has a general role independent of the iron status, whereas HapX function is confined to iron depletion. In agreement, deficiency in any of the CBC subunits has deleterious

consequences not only during iron depleted conditions, as with HapX ablation, but also during iron-replete growth. Consistently, expression of HapX is repressed by iron whereas that of the CBC subunit HapC is constitutive. Moreover, deficiency in HapC but not HapX causes up-regulation of iron-dependent pathways also during iron-replete conditions.

334. G-protein alpha subunit Gpa3 regulates sexual differentiation in *Cryptococcus neoformans.* Kung-Hung Liu[1] and Wei-Chiang Shen[2]. Department of Plant Pathology and Microbiology, National Taiwan University, Taiwan. [1]gloclovlo@yahoo.com.tw [2]wcshen@ntu.edu.tw

The conserved G-protein signaling molecules play pivotal roles in transducing the environmental stimuli to trigger appropriate cellular responses. Extensive studies demonstrated that diverse developmental processes and pathogenesis in fungi are intricately regulated by the activities of G-proteins. In *Cryptococcus neoformans*, an opportunistic human fungal pathogen, three G-protein alpha subunits were identified. Gpa1 was shown to act at the upstream of the cAMP response pathway to regulate sexual reproduction and virulence. Here, we generated and characterized two other G-protein alpha subunit mutants, *gpa2* and *gpa3* . In the *gpa2* mutant, no discernible phenotype was observed under all conditions tested. In contrast, elevated level of sexual filamentation was observed in the *gpa3* mutant, especially more pronounced in the monokaryotic fruiting process. Deletion of the heterotrimeric G-beta subunit *GPB1* in the *gpa3* mutant background blocks mating differentiation as the *gpb1* mutant. Our results demonstrated that G-protein alpha subunit Gpa3 and beta subunit Gpb1 are integral components of a heterotrimeric G-protein complex which function upstream of the pheromone response pathway to regulate sexual differentiation in *C. neoformans*.

335. Establishing new vectors for transformation and gene-silencing in *Botryis cinerea*. Risha Patel, Gary Foster and Andy Bailey. University of Bristol, Biological Sciences, Bristol, UK.

***Botrytis cinerea* is a necrotrophic fungus capable of infecting over 300 plant species worldwide. Typically, *Botrytis cinerea* is referred to as grey mold rot or Botrytis blight. It normally attacks various damaged or cracked plant tissues including leaves, weeds, stems, and seeds. In general, *B. cinerea* becoming more resistant to current fungicides. As a result, agrochemical companies are seeking new targets capable of minimizing pathogenicity of *B. cinerea*. The use of gene silencing is a well-established technique in animal, plant and fungal systems. The benefits of down regulating genes are vast, including the potential to identify protein function of targeted genes. An effective gene silencing model must be demonstrated and developed before useful targets may be identified. In our initial investigations, we utilized the widely used vector pLOB1 and derivatives thereof to transform and trigger silencing of laccase and superoxide dismutase in *Botrytis*. Silencing was successful, with low to intermediate levels of gene silencing observed for both target transcripts, however we also identified unexpected reductions in virulence that did not correlate with the targeted transcript. Investigation shows that these were due to specific sequences within the pLOB1 plasmid and for this reason we strongly recommend that researchers move to a different construct for transformation-based experiments. New transformation and expression vectors have been constructed containing the *A. nidulans oliC* promoter and *trpC* terminator. These completely alleviate the difficulties observed with pLOB1. The new vector system displayed increased transformation rates and silencing efficiencies without the detrimental effects or reduction in pathogenicity observed with the pLOB1 system. We have strong evidence that gene silencing is possible within *Botrytis cinerea*, giving transformants displaying a range of silencing levels and this may be of great benefit when used to assess the amount of a particular gene product necessary for the infection process rather than the all-or-nothing studies achieved through targeted gene disruption.**

336. The *Fusarium oxysporum fost12* Gene Involved in Virulence Is Upregulated *in planta.* M. A. García-Sánchez, M. Brisa-Ramos, N. Martín-Rodrígues , J.J. De Vega-Bartol, A. P. Eslava and J. M. Díaz-Mínguez . Centro Hispano-Luso de Investigaciones Agrarias (CIALE) Universidad de Salamanca 37007, Salamanca, Spain Phone Fax: +34 923 294663 chonela@usal.es , josediaz@usal.es

***Fusarium oxysporum* is an ubiquitous plant pathogen with a broad host range; however the isolates are restricted in their host specificity and may be classified into *formae speciales*. This feature makes this plant pathogen an atractive model for the study of gene expression patterns involved in patogenicity and virulence. Several studies have demonstrated that the mitogen-activated protein (MAP) kinase signal transduction pathways play a rol in fungal pathogenesis. Previous results have shown that *fost12* is involved in virulence. The quantification of *fost12* transcript produced *in planta* by a highly virulent strain, as measured by Real Time PCR, showed little accumulation of *fost12* transcript in root and a dramatic increase in stem during the early stages of plant infection. We have also investigated the influence of ambient pH and nutrient starvation in the expression of *fost12* when the fungus grows in culture media. No clear correlation between pH and differences in the accumulation of *fost12* transcript could be observed, thus indicating that changes in pH alone are not important in the regulation of the gene. On the contrary, starvation conditions (either the lack of a carbon or nitrogen source) affect the accumulation of *fost12*, as indicated by the Northen analysis results. Interestingly, the *fost12* promoter shows motifs involved in the regulation by glucose or nitrogen, but no binding motif for the pH regulator PacC was**

found. We conclude that the upregulation of *fost12 in planta* seems to be induced by the scarce availability of nutrients, at least in the early stages of infection.

337. Regulation of *Trichoderma virens MRSP1*, encoding a novel secreted protein. Ella Pardovitz-Kedmi[1], Ada Viterbo[2], Yael Mandel-Gutfreund[1],Ilan Chet[2], Prasun K. Mukherjee[3], and Benjamin A. Horwitz[1]. [1]Biology, Technion, Haifa, Israel; [2]Biochemistry, Weizmann Institute of Science, Rehovot, Israel, [3]Nuclear Agriculture and Biotechnology Division, BARC, Mumbai, India.

***MRSP1* (MAP kinase Repressed Secreted Protein 1) is highly overexpressed in the *tmkA* MAP kinase mutant of the biocontrol fungus *Trichoderma virens* [1]. Transcript levels of *MRSP1* are determined by presence or absence of TmkA, rather than by light or sporulation, making it a molecular marker for the unusual, negative, regulation by TmkA. The predicted protein is 15.9 kDa, has a secretory signal, shows some homology to plant expansins, and contains a four-cysteine pattern C-X29-CP(G)C-X31-C which defines a new fungal cysteine-rich motif [1]. The tight regulation by TmkA, and presence of homologs in other fungal genomes, suggests that MRSP1 may have important functions, but the low expression level in wild type, in axenic culture, raises questions about when the protein acts. As an initial test of whether MRSP1 might be involved in interaction between *T. virens* and plant roots, we are following its expression during contact of the fungus with cucumber roots and penetration into cortical layers. [1] P.K. Mukherjee, R. Hadar, E. Pardovitz-Kedmi, N. Trushina and B.A. Horwitz, Biochem. Biophys. Res. Commun. 350(2006) 716–722.**

338. Characterization of the fumonisin biosynthetic regulatory gene *FUM21* and multiple alternative splice forms. Daren W. Brown, Robert A.E. Butchko, Mark Busman and Robert H. Proctor. Mycotoxin Research Unit, U.S. Department of Agriculture-ARS, Peoria, IL 61604. e-mail: browndw@ncaur.usda.gov

Fumonisins are a family of mycotoxins produced by some *Fusarium* species and can contaminate maize or maize products. Ingestion of fumonisins is associated with diseases, including cancer and neural tube defects, in humans and animals. In fungi, genes involved in synthesis of mycotoxins and other secondary metabolites are often located adjacent to each other in gene clusters. The fumonisin biosynthetic gene cluster contains 17 genes of which one, *FUM21*, encodes narrow domain transcription factor required for fumonisin biosynthesis. The Fum21p is predicted to include a Zn(II)2Cys6 DNA binding domain and a second domain associated with fungal transcription factors. Analysis of *FUM21* cDNAs identified four alternative splice forms (ASFs) and microarray analysis indicated the ASFs were differentially expressed. Based on these data, we present a model for how *FUM21* ASFs may regulate fumonisin biosynthesis.

339. Disruption of *TUP1* in H99 reveals its role in capsule formation and virulence in *Cryptococcus neoformans*. Hyeseung Lee, Yun C. Chang, and K.J. Kwon-Chung. LCID, NIAID, NIH, Bethesda, MD 20892, USA.

***Cryptococcus neoformans*, the etiologic agent of cryptococcosis is divided into two species *C. neoformans* (serotype A and D) and *C. gattii* (serotype B and C). Serotype A strains are world-wide in distribution while the other serotypes are geographically more restricted. Although H99 (serotype A) and JEC21(serotype D), the representative strains of serotype A and D, share 95% identity in genome sequence, various serotype-specific pathobiological differences have been observed. Previously, we showed that *TUP1* disruption resulted in mating type-specific phenotypes in JEC21 derived strains. Subsequent study revealed a quorum sensing-like phenomenon in *tup1Δ* of both mating types in serotype D strains. In this study, we have deleted *TUP1* from the H99 strain to study the scope of its regulatory role in a serotype A strain. Mating efficiency of H99 *tup1Δ* was severely reduced as seen in serotype D *tup1Δ* strains. Unlike serotype D strains, however, H99 *tup1Δ* showed no quorum sensing-like phenotype and showed growth retardation not limited to 25°C and 30°C. Interestingly, in contrast to the serotype D *tup1Δ*, H99 *tup1Δ* produced much larger capsule than the wild type regardless of media. Transcriptional profiling revealed that expression of many genes related to iron homeostasis was affected by *TUP1* deletion in H99, possibly explaining the hypercapsular phenotype in H99 *tup1Δ*. In spite of the hypercapsule in vitro, H99 *tup1Δ* strain had attenuated virulence in mice compared to wild type strain. Capsule size of the yeast cells in brain smears from mice infected with wild type was similar to those infected with the deletant. Taken together, the global regulator *TUP1* plays both conserved and distinct roles in serotype A and D strains.**

340. Applying PARAFAC models to detect new regulatory mechanisms in *Fusaria*. Jens A. Andersson[1], Claus A. Andersson[2], Rasmus Frandsen[1]. [1]Institute of Ecology, Genetics and Microbiology, University of Copenhagen [2]Spectroscopy and Chemometrics group, University of Copenhagen

High performance liquid chromatography (HPLC) analysis offers an easy, cost-efficient and reliable method for detection of fungal metabolites. However, subsequent data-analysis is often time consuming, narrow and dependent on experienced personnel to recognise characteristic UV-spectra. Here a mathematically based method for detecting metabolites and classification of a number of *Fusaria*-strains belonging to the species *F. graminearum*, *F. pseudograminearum* and *F. culmorum* based on multiway analysis of HPLC-data is presented. By applying a PARAFAC-model in the analysis of 3-

dimensional HPLC data it is possible to detect characteristic metabolite patterns within and between species. Characteristic metabolite profiles can serve as an efficient tool for chemotaxonomic classification of *Fusaria* species. Analysis of correlation between synthesized compounds within and between species of *Fusaria* may disclose new constituents in the regulatory networks that are known to control the synthesis of secondary metabolites. As expected, the models clearly indicate that the diversity of synthesized metabolites differ between strains, species and according to growth media composition. By linking fungal metabolite profiles with expression patterns of known polyketide synthase (PKS) and non ribosomal peptide synthase (NPS)-genes it is here postulated, that a 3-way analysis may serve as an efficient tool to link genotype to fenotype.

341. Functional characterization of a MAPK cascade in *Neurospora crassa*. G. Park and K.A. Borkovich. Department of Plant Pathology, University of California, Riverside, CA 92521

Mitogen-Activated Protein (MAP) kinase cascades are composed of MAP kinase kinase kinases (MAPKKK), MAP kinase kinases (MAPKK) and MAP kinases (MAPK). These systems are conserved in filamentous fungi. Three components of a MAPK cascade (*mik-1*, MAPKKK; *mek-1*, MAPKK; *mak-1*, MAPK), homologous to that controlling cell wall integrity in *Saccharomyces cerevisiae* were annotated in the *Neurospora crassa* genome sequence. Targeted gene replacement of these three kinases (*mik-1*, *mek- 1*, and *mkc-1*) was performed by the *Neurospora* genome project. In this study, we characterize the function of this MAPK cascade using these mutants. *mik-1*, *mek-1*, and *mak-1* knockout mutants exhibited significant reduction in apical extension of basal hyphae on solid medium. All three mutants formed short aerial hyphae and arthroconidia formation was observed in all three mutants.. Macroconidiation was almost abolished in both *mek-1* and *mkc-1* knockout mutants, whereas the *mik-1* knockout strain produced reduced levels of macroconidia in comparison to wild type. All three mutants were unable to form protoperithecia or perithecia when used as females in a sexual cross. We are currently analyzing levels of *MAK-1* phosphorylation and effects on gene expression in the three mutants during growth and development in *N. crassa*.

342. Transcription Factors and Chromatin Remodeling in the Neurospora Clock. William Belden, Jennifer Loros, Jay Dunla.p Department of Genetics Dartmouth Medical School Hanover 03755, USA

Positive and negative transcriptional/translation feedback loops involving the frequency gene are central to circadian rhythms in *Neurospora crassa*. In this model, the transcription factors White Collar-1 (WC-1) and White Collar- 2 (WC-2) activate frq expression in a circadian and light dependent manner; they are found together and have been assumed to act exclusively as a heterodimeric complex. Surprisingly however, chromatin immunoprecipitations (ChIP) indicate that the WC proteins do not act solely as an obligate complex: in vivo binding of WC-2 to the *frq* promoter occurs in a rhythmic fashion with the peak binding occurring coincident with the peak in *frq* transcription, whereas WC-1 is bound continuously with only slight increases observed when *frq* is being transcribed. Nuclease accessibility experiments show chromatin rearrangement at the *frq* promoter when the gene is expressed. To identify the enzymes mediating the rearrangement, all 19 Neurospora genes with homology to the *swi2/ snf2* family of ATP-dependent chromatin-remodeling enzymes were deleted by gene replacement. Examination of these strains identified a gene, designated *clockswitch (csw-1)*, required for normal clock function. The data suggest a role where CSW-1 is required for disassembly of active WCC at *frq* and is needed for the sharp transition from the transcriptionally active phase to the repressed state.

343. Identification of genes regulated during asexual development of *Neurospora crassa*. C. Greenwald, T. Kasuga, L. Glass, B. D. Shaw, D. J. Ebbole and H. H. Wilkinson. Texas A&M University, Plant Pathology and Microbiology, College Station, TX and University of California, Berkeley.

We are interested in identifying gene networks associated with asexual development across the filamentous fungi. As a first step toward this goal we have used the full genome microarray available for *Neurospora crassa* to profile the mRNAs produced during 1) a developmental time course (0, 2, 4, 8, 10, 12, 14, 18, 24 hours), and 2) a comparison of mycelial vs. aerial tissue at 12 hours. Analysis of variance (ANOVA) was used to discern differences among time points. It yielded >3300 significantly regulated genes, representing a third of the >10,000 *N. crassa* genes on the microarray. Analysis to establish the major categories of expression patterns present among these significantly regulated genes is ongoing. For example, a set of genes known to be expressed during conidiation (*con-10, con-6, eas*) were regulated as expected (induced specifically in aerial tissue). A total of 91 genes with similarity (0.95 Pearson's coefficient) to at least one of several of these known conidiation-specific genes have been discovered with these experiments. Further experiments to evaluate the expression of these candidate genes in *N. crassa* developmental mutants (e.g. *fl*) are underway.

344. A gene with homology to the Cytochrome P450 conserved functional domain is down-regulated during sexual development in *Schizophylum commune.* Charles McEntee[1], Lucas Bernacki[1], and Kirk Bartholomew[1*]. [1]Department of Biology, Sacred Heart University, Fairfield, CT USA; bartholomewk@sacredheart.edu [*]Corresponding Author

The well-characterized A and B mating-type loci of *Schizophylum commune* control activation of sexual development when haploid mycelia of non-self mating-type interact. The protein products of both the A and B loci are proposed to initiate the events of sexual development by controlling the expression of target genes in compatible mates eventually leading to the formation of the dikaryotic mycelia capable of progressing through sexual reproduction. A previous study using the reverse transcriptase differential display polymerase chain reaction method to isolate genes expressed differentially during the early stages of sexual development resulted in the cloning and sequencing of ddPCR04_05 10b, a partial cDNA with significant homology to the cytochrome p450 family of proteins (pfam00067). We have confirmed the down-regulation of ddPCR04_05 10b at the 48 hour time-point post-mating by quantitative reverse transcriptase PCR. Current progress in cloning, sequencing, and functional analysis is reported.

345. A novel Zn_2Cys_6 transcription activator, AtrR, regulates ABC transporter genes conferring azole drug resistance in *Aspergilli.* Daisuke Miura, Katsuya Gomi. Graduate School of Agricultural Science, Tohoku University, Sendai, Japan.

We have already found that the expression of three genes that each encodes ABC transporter (*AoatrA, AoatrF, AoatrG*) was highly upregulated in a spontaneous mutant of *Aspergillus oryzae* showing resistance to azole fungicides. Since the expression of several ABC transporter genes was upregulated simultaneously in the mutant, we assumed that azole resistance is caused by mutation of a common transcription factor that controls these gene expressions. Promoter deletion analyses of the *AoatrA* suggested that a conserved sequence homologous to the PDR1/PDR3-response element (PDRE) is required for gene expression of ABC transporters. A BLAST search of *A. oryzae* genome database revealed the existence of several genes with a relatively high similarity to Zn_2Cys_6 motif of PDR1/PDR3. Overexpression of one of these genes under the control of the *amyB* promoter resulted in increased drug resistance and also affected the expression level of ABC transporter genes in *A. oryzae*. In addition, both strains with deletion of this regulatory gene in *A. oryzae* and with its ortholog deleted in *A. nidulans* were hypersensitive to azoles. These results indicate that the transcription factor, designated AtrR, regulates gene expression of ABC transporters that would function as drug efflux pumps in *Aspergilli*.

346. Transgene-induced silencing of the zoosporogenesis-specific *PiNIFC* gene cluster in the oomycete *Phytophthora infestans* involves chromatin alterations. Howard S. Judelson and Shuji Tani, University of California, Riverside, California, USA

Transformation-based methods for silencing genes in oomycetes are still in their infancy and the underlying cellular mechanisms are poorly understood. Transformants of the phytopathogen *Phytophthora infestans* in which the *PiNIF* family of transcriptional regulators were silenced were therefore generated and analyzed to better understand the process. *PiNIFC1*, *PiNIFC2*, and *PiNIFC3* are zoosporogenesis-induced and clustered within 4 kb, and 20 kb away resides a sporulation-induced form, *PiNIFS*. Hairpin constructs of *PiNIFC1* or *PiNIFC2* triggered silencing of the cognate gene in about one-third of transformants and frequently all three *PiNIFC* genes became silenced coordinately. This resulted in a defect in the germination of zoospore cysts. *PiNIFS* was not silenced by the *PiNIFC* transgenes, even though all genes are closely related in DNA sequence. Silencing of the *PiNIFC* cluster was transcriptional based on nuclear run-on assays, and associated with tighter chromatin packing based on nuclease accessibility experiments involving DNA blotting and qPCR. The chromatin alterations extended a few hundred nucleotides beyond the boundaries of the transcribed region of the *PiNIFC* cluster, and were not associated with increased DNA methylation. Silencing of the different members of the *PiNIFC* family by a spreading heterochromatin domain, as opposed to a diffusible signal, may explain why PiNIFS escaped silencing.

347. Involvement of OS-2 MAP kinase pathway in regulation of fungal-specific catalases in *Neurospora crassa.* Kazuhiro Yamashita[1], Azusa Shiozawa[1], Shinpei Banno[2], Fumiyasu Fukumori[1], and Makoto Fujimura[1]. [1]Life Sciences, Toyo University, Itakura, Gunma, Japan. [2] Plant Function Research Center, Toyo University, Itakura, Gunma, Japan.

***Neurospora crassa* has three fungal specific catalases, CAT-1 (conidia-specific), CAT-2 (peroxisomal catalase), and CAT-3 (hypha-specific). Transcription of the *cat-2* and *cat-3* genes was stimulated by heat shock and oxidative stress, respectively. In contrast, expression of the *cat-1* gene was significantly stimulated by osmotic stress and fludioxonil in the wild-type strain, but not *os-2* MAP kinase mutant. Active staining of catalase after native electrophoresis demonstrated that conidia of the *os-2* mutant contained low level of CAT-1 protein. These results indicated *cat-1* gene expression was regulated by OS-2 MAP kinase. Additionally, the *os-2* conidia were sensitive to oxidative stress than those of the wild-type stain, probably due to the low mount of CAT-1 catalase. Fludioxonil, an activator of OS-2 MAP kinase, induced the conidia specific CAT-1 activity in hyphae of the wild-type strain. Interestingly, this fungicide drastically decreased hypha-specific CAT-3 activity, although impaired activation of CAT-1 and inactivation of CAT-3 was not observed in the hyphae treated by osmotic stress. These results suggest that OS-2 MAP kinase may play an important rule for asexual differentiation through the post-transcriptional regulation of fungal-specific catalase expression.**

348. *Aspergillus oryzae atfA* Encodes a Transcription Factor, which is Required for Vigorous Growth in the Solid-State Fermentation. K. Sakamoto[1], O. Yamada[1], Y. Okita[1], K. Iwashita[1], O. Akita[2], K. Gomi[3], S. Mikami[1]. [1]National Research Institute of Brewing, 3-7-1 Kagamiyama Higashi-Hiroshima, Hiroshima, Japan. [2]Jissen Women's University, 4-1-1 Osakaue, Hino, Tokyo, Japan. [3]Tohoku University, 1-1 Tsutsumidori-Amamiyatyou Aoba Sendai, Miyagi, Japan

In the solid-state culture, *Aspergillus oryzae* exhibits phenotypes such as the high production of enzymes and conidiophore development. 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 a gene encoding transcription factors that show high homology to *atf1* of *Shizosaccharomyces pombe* and named it *atfA*. We constructed *atfA*-deletion strain (DelA51) to analyze the function. The germination ratio of DelA51 conidia was reduced to 13.7%, while that of wild type (wt) conidia was over 90 %. Furthermore the DelA51 conidia were more sensitive to stress than wt. Although DelA51 grew as fast as wt on the agar plate or in the submerged liquid culture, the growth of DelA51 was delayed in solid-state fermentation or on the hyper-osmotic agar plate. Furthermore high humidity of atmosphere restored the growth of DelA51. Thus we concluded that *atfA* is necessary for the vigorous growth on the low water activity substrate. To determine the genes regulated by *atfA*, gene expression of DelA51 was compared with that of wt using microarrays under osmotic stress condition and we identified 34 genes down-regulated in DelA51.

349. The expression of the novel sexual developmental activator, *nsdC*, in *Aspergillus nidulans* is regulated by complex transcriptional and post-transcriptional control. Hye-Ryun Kim, Yong-Woo Shin and Dong-Min Han Dept. of Life Science, Wonkwang University, Korea 570-749

The *nsdC* gene predicted to encode a putative transcription factor carrying a novel type of zinc fingers consisting of two C2H2 and a C2HC motifs plays as a positive regulator of sexual development in *A. nidulans*. Two distinct transcripts, 3.2 and 2.8 kb in size, are synthesized and the smaller one differentially accumulates in various stages of growth and development as well as under the varying cultural conditions. It increases in 10 min after the exposure of hypoxically cultured or carbon starved mycelia to air or glucose, respectively, and is restored to normal level in following 10 min. The smaller mRNA is maintained to increased level in *creA204* mutant strain. The results suggest that the alternative transcription of *nsdC* mRNA is closely related with carbon metabolism. 5' RACE and Northern blot analysis revealed the presence of more than two alternative transcription initiation sites which might be responsible for the generation of two kinds of transcript in different size. Two alternative transcription termination sites were also identified through 3' RACE and Northern blot analysis of the *nsdC* deletion mutant. The gene carries two relatively long introns in its 5'UTR, one of which is spliced alternatively. Taken together, our studies suggest that the expression of *nsdC* is controlled by complex transcriptional and post transcriptional regulations in response to the state of carbon metabolism.

350. Withdrawn

351. Modulation of the entrained phase of the circadian clock of Neurospora by large and small isoforms of FREQUENCY. Axel Diernfellner[1], Hildur V. Colot[2], Orfeas Dintsis[1], Jennifer J. Loros[2], Jay C. Dunlap[2] and Michael Brunner[1]. [1]University of Heidelberg Biochemistry Center, INF 328, 69120 Heidelberg, Germany; [2]Departments of Genetics and Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755, USA

The large (l) and small (s) isoforms of the circadian clock protein Frequency (FRQ) of Neurospora are parts of a negative feedback loop repressing transcription of their common gene. The ratio of expression of l-FRQ vs. s-FRQ is regulated by temperature dependent splicing. Strains that express only l-frq support display temperature-compensated circadian rhythms whose periods are shorter than those of strains expressing only s-frq. The free-running period of wt decreases slightly with increasing temperature, reflecting the expression ratio of l- vs. s-FRQ. s-FRQ appears to be a significantly better repressor than l-FRQ. In 24 h LD cycles l-frq entrains to an earlier and s-frq to a later phase than wt. The entrained phases depend on day length and temperature. In contrast, the entrained phase of wt is essentially independent of both parameters.

352. Characterisation of the *os* signal transduction pathway in *Botrytis cinerea.* Weiwei Liu, Muriel Viaud, Pierre Leroux and Sabine Fillinger. UMR BIOGER, INRA Versailles, Route de Saint-Cyr, 78026 Versailles cedex, France

***Botrytis cinerea*, a necrotrophic fungal pathogen, has to defence itself against high oxidative and osmotic condition *in planta*. In fungi, the best studied case of signal transduction is the HOG pathway of *Saccharomycese cerevisiae* which is required to respond to high osmolarity and oxidative stress. In addition, the homologous *os* pathway in *Neurospora crassa* was demonstrated to be involved also in resistance to fungicides. Interested to understand the function of the *os* pathway in adaptation to adverse environmental conditions, pathogenesis and certain fungicides, we have studied this pathway in the phytopathogenic fungus *B. cinerea* via the characterization of the principal components, the histidine kinase Bos1 and the MAP kinase Sak1. *BOS1* inactivation had pleiotropic effects in *B. cinerea*: abnormal morphology, loss of**

macroconidiation, osmosensitivity, sensitivity to some oxidative stress conditions, resistance to three classes of fungicides and loss of virulence. On the other hand, the Δ*sak1* mutant showed the same phenotypes as the Δ*bos1* mutant with a single exception: no resistance to the fungicides was observed in the Δ*sak1* mutant, suggesting that another pathway independent from MAP kinase Sak1 via histidine kinase Bos1 could be required for fungicide resistance in *B. cinerea*. The Δ*bos1* Δ*sak1* double mutant showed resistance to the fungicides as the Δ*bos1* single mutant, confirming that the MAP kinase Sak1 is not directly involved in resistance to these fungicides. Another interesting feature of the *os* signal-transduction pathway is that the regulation of Sak1 operates at different levels, on the mRNA level and through phosphorylation.

353. Survey of Genome Wide Occurrence of Alternative Splice Forms in *Fusarium verticillioides.* Daren W. Brown[1], Robert A. E. Butchko[1], and Li-Jun Ma[2]. [1]Mycotoxin Research Unit, U.S. Department of Agriculture-ARS, Peoria, IL 61604 [2]The Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA, 02141

***Fusarium verticillioides* is a pathogen of maize and synthesizes the fumonisin family of mycotoxins. Contamination of infected kernels with fumonisins contribute significantly to economic losses to the maize grain industry worldwide. Ingested fumonisins cause a variety of animal diseases and are correlated to human cancers and neural tube defects. Understanding basic fundamental strategies regulating fungal processes will aid our efforts to limit *F. verticillioides* maize diseases as well as fumonisin contamination. Alternative splicing of transcripts in many eukaryotes play a critical role in expanding gene function by increasing protein diversity. The few examples of alternative splice forms (ASFs) described in fungi predominantly involve intron retention. Analysis of large-scale expressed sequence (EST) collections coupled with genomic DNA from a number of fungi indicate that ASFs are more widely prevalent than previously thought. Analysis of over 87,000 *F. verticillioides* ESTs provided evidence of ASFs for over 300 genes which represent almost 3.1% of the genes detected. Although a majority of ASFs retain introns, we present evidence for a variety of alternative splice mechanisms. These results clearly show that alternative splicing in fungi is more common than previously thought and likely play a significant role modifying gene function by increasing the transcript diversity.**

354. L-arginine induces structural and functional changes of the mRNA in *Aspergillus nidulans.* Borsuk P[1], Przykorska A[2], Blachnio K[2], Koper M[1], Pekala M[1] and Weglenski P[1]. [1]Institute of Genetics and Biotechnology, University of Warsaw [2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences

Expression of arginase gene (*agaA*) in *Aspergillus nidulans* depends on growth conditions, especially on the presence or absence of arginine and various nitrogen and carbon sources in the medium. 5'UTR of arginase mRNA contain arginine binding motifs, short open reading frame and intron with two putative 3' splice site. We present evidence that: - L-arginine binds *in vitro* directly and stereospecifically to the arginase 5'UTR - L-arginine binds to the 5'UTR at more then one site - only L-arginine (not several other L-amino acids or D-arginine) generates drastic changes in the secondary structure of the 5'UTR - putrescine (poliamine) has no influence on the secondary structure of the 5'UTR - L-arginine and poliamines forces the selection of one of the two 3' splice sites in the intron present in the 5'UTR We postulate that expression of the *agaA* gene is regulated at both transcriptional and posttranscriptional levels. Posttranscriptional regulation depends on mRNA stability affected by the riboswitch-mediated alternative splicing in the 5'UTR. The role of poliamine in control of *agaA* expression is also discussed.

355. Control of Translation and mRNA Stability by the Fungal Arginine Attenuator Peptide. Christina C. Spevak, Heather M. Hood, Jaime McCollum, and Matthew S. Sachs, Oregon Health & Science University, 20000 NW Walker Road, Beaverton, OR 97006-8921

Fungal mRNAs specifying the small subunit of Arg-specific carbamoyl phosphate synthetase contain an upstream open reading frame (uORF) specifying the evolutionarily conserved arginine attenuator peptide (AAP). The synthesis and amino acid sequence of the AAP are critical for cis-acting Arg-specific negative regulation of translation. In cell-free translation systems, AAP-mediated regulation depends on the AAP's ability to stall ribosomes at the uORF termination codon in response to arginine. Certain single missense mutations eliminate ribosome stalling in vitro and Arg-specific regulation in vivo and in vitro. The S. cerevisiae CPA1 and N. crassa AAPs are specified by uORFs whose start codons are in relatively poor translation initiation contexts, resulting in much leaky-scanning past the uORF when Arg levels are low, with no stalling of ribosomes on the uORF when it is translated. When Arg is high, AAP-mediated ribosome stalling blocks ribosomes that scanned past the uORF initiation codon from initiating translation at the downstream start codon. The AAP sequences required for stalling were determined by Ala- and Pro-scanning and by analyzing the minimal domain required to induce stalling. The minimal domain contains the most highly conserved region among yeasts, ascomycetes and basidiomycetes. In addition to translational regulation, CPA1 expression is naturally regulated at the level of mRNA stability via a mechanism involving nonsense-mediated mRNA decay (NMD). Arg-regulated stalling at the CPA1 uORF stop codon triggers NMD and the data suggest a simple model that controlling the density of ribosomes at the uORF termination codon modulates NMD. Analyses of N. crassa arg 2 in isogenic NMD+ and nmd- strains indicate

that its levels are also controlled by NMD. Thus, these studies provide a basis for a general understanding of how ribosome occupancy of a uORF termination codon controls both translation and mRNA stability in eukaryotes.

356. Cloning and Characterization of Circadian Output Pathway mutant COP1-4. Vitalini M.W. and D. Bell-Pedersen. Texas A& M University.

Decades of research on the *Neurospora crassa* circadian clock have resulted in a detailed understanding of core components of the central FRQ/WCC oscillator. However, in *Neurospora*, as well as in the other model systems, little is known about the pathways by which temporal information is relayed from a circadian oscillator to the rest of the cell to control downstream rhythmic processes. We have set up a genetic selection for clock mutations in order to identify novel components involved in output from the *Neurospora* circadian clock. This selection is based on the differential expression from the promoters of clock-controlled genes (ccg) fused to a selectable marker (*mtr*) in response to the presence or absence of clock gene product FRQ. After screening hundreds of mutant colonies we have narrowed our focus to a few carefully chosen mutations that disrupt the rhythmic expression of more than one ccg. We found that two genes, when mutated, affect circadian output and are involved in the ability of cells to respond to hyper- osmotic conditions. We will present the cloning and further characterization of one of these mutations, cop1-4, and discuss the link between circadian output and stress-response pathways.

357. Withdrawn

Developmental Biology

358. Role of putative apoptosis-inducing factor (AIF)-homologs in lifespan control of the filamentous ascomycete *Podospora anserina*. Diana Brust, Andrea Hamann, Heinz D. Osiewacz Institute of Molecular Biosciences, J.W. Goethe-University, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany E-mail: brust@bio.uni-frankfurt.de

Wild-type strains of the ascomycete *Podospora anserina* are characterized by a limited life span. After about 30 days of linear growth, the growth rate of the wild type strain s declines and the morphology and physiology of cultures changes dramatically, ending up with the death of the hyphal tips. This process has a clear mitochondrial etiology. The role of mitochondria in aging of cultures raises the question whether or not apoptosis-like processes are involved in the final stage of development of this fungus. An in-silico analysis identified several putative apoptosis factors. Especially the AIF (apoptosis-inducing factor) and AMID (AIF homologous mitochondrion-associated inducer of death) homologs are of great interest since the mammalian proteins are known to be involved in a mitochondrial pathway of apoptosis. The PaAIF1/GFP fusion protein was found to be localized in the cytoplasm, while the PaAMID2/GFP fusion protein localizes to mitochondria suggesting a role in a mitochondria-dependent and caspase-independent signalling pathway. The localization of PaAMID1 is under investigation. Deletion of *PaAmid1* results in a moderate lifespan extension on complete medium (59 %), while the *PaAif1::ble* strain is characterized by a mean lifespan of 150 days (340 % increase) on this medium. Interestingly, the double deletion strain *PaAmid1::ble, PaAif1::ble* is strongly impaired in fertility. Currently, we investigate the age-dependent translocation of the *P. anserina* AIF and AMID homologs and the mitochondrial release after an apoptotic stimulus achieved by chemical inductors.

359. The NADPH oxidases NOX-1, NOX-2 and their regulatory subunit NOR-1 regulate growth and differentiation in *Neurospora crassa*. Nallely Cano; Karen Álvarez; Wilhelm Hansberg and Jesús Aguirre. Instituto de Fisiología Celular,UNAM. Mexico. ncano@ifc.unam.mx

We have proposed that reactive oxygen species(ROS) play essential roles for cell differentiation in microbial eukaryotes. The NADPH oxidases (NOX) are enzymes that catalyze the production of the superoxide. In phagocytic cells, the NOX consists of the membrane-associated catalytic core gp91phox and p22phox subunits (cytochrome b558). The assembly of the cytosolic regulatory proteins p47phox, p67phox, p40phox and Rac1/Rac2 with the cytochrome b558 results in NOX activation. NoxA from *A. nidulans*, the gp91phox microbial orthologue, was found to produce ROS and to be essential for differentiation of sexual fruiting bodies (Lara-Ortiz et al., 2003). p67phox homologues have been reported in *Dictyostelium discoideum* (Lardy et al., 2005) and *Epichloë festucae* (Takemoto et al., 2006). In *E. festucae*, this subunit (NoxR) regulates Nox1 during symbiosis. Here we show that inactivation of NOX-1 in *N.crassa* results in decreased asexual development, a slight reduction of radial growth and complete female sterility. Mutation of NOX-2 did not affect any of these processes but led to the production of ascospores that failed to germinate even in the presence of hydrogen peroxide or furfural. Mutation of the gp67phox homologue NOR-1 resulted in reduction of asexual development and radial growth, female sterility and lack of ascospore germination. These results suggest that NOR-1 regulates both, NOX-1 and NOX-2, at different developmental stages.

360. Effect of Ras signaling on fruitbody formation in the fungus *Schizophyllum commune.* Nicole Knabe, Daniela Schubert & Erika Kothe. Friedrich-Schiller-University Jena, Dept. of Microbiology, Neugasse 25, 07743 Jena, Germany, Nicole.Knabe@uni-jena.de

The involvement of small monomeric G-proteins like Ras has been shown in different fungi for MAPK cascade signaling and/or cAMP-dependent pathways. In addition, cAMP signaling has been implicated in fruitbody development. The involvement of Ras in sexual development and cell morphogenesis was investigated by characterization of *ras1* and *gap1* genes in *S. commune*. Disruption of *gap1* encoding a Ras-dependent GAP (GTPase activating protein) leads to accumulation of Ras in its activated, GTP-bound state and to constitutive Ras signaling. The phenotype of *gap1* deletion strains shows disorientated growth pattern. Dikaryons are not able to produce normal clamp connections because the 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. *Deltagap1/Deltagap1* dikaryons formed increased numbers of fruitbody primordia, whereas the amount of fruitbodies was not raised. Mature fruitbodies formed no or abnormal gills. No production of spores could be observed. To further analyse the function of Ras, amino acid mutations in the GTP-binding region and GTPase domains were introduced *in vitro*. The phenotypes of the resulting transformants carrying the dominant negative allele Ras^{G15N} and the constitutive alleles Ras^{G12V} and Ras^{Q61L} were examined. Similar phenotypes of the RasGap deletion strain and the constitutive Ras mutant could be observed. Both shows a reduced

361. Transcriptional and post-transcriptional levels of control lead to a fruiting body-specific accumulation of the APP protein in *Sordaria macrospora* and *Neurospora crassa.* 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, email: minou.nowrousian@ruhr-uni-bochum.de

During fungal fruiting body development, specialized cell types differentiate from vegetative mycelium. We have isolated a protein from the ascomycete *Sordaria macrospora* that is not present during vegetative growth but accumulates in perithecia. The protein was sequenced by mass spectrometry and the corresponding gene was termed *app* (abundant perithecial protein). *app* transcript occurs only after the onset of sexual development; however, the formation of ascospores is not a prerequisite for APP accumulation. Fruiting body-specific accumulation of an APP-EGFP fusion protein is dependent on regulatory regions upstream and downstream of the *app* open reading frame. The transcript of the *N. crassa* ortholog is present prior to fertilization, but the protein accumulates only after fertilization. In crosses of *N. crassa* delta-app strains with the wild type, APP accumulates when the wild type serves as female parent, but not in the reciprocal cross; thus, the presence of a functional female *app* allele is necessary and sufficient for APP accumulation. These findings highlight multiple layers of temporal and spatial control of gene expression during fungal development (Fungal Genet Biol, 2006, in press).

362. The novel ER membrane protein PRO41 is essential for sexual development in *Sordaria macrospora.* Minou Nowrousian[1], Sandra Frank[1], Sandra Koers[1], Peter Strauch[1], Thomas Weitner[1], Carol Ringelberg[2], Jay C. Dunlap[2], Jennifer J. Loros[2], and Ulrich Kück[1]. [1]Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, 44801 Bochum, Germany, and [2]Departments of Genetics and Biochemistry, Dartmouth Medical School, NH 03755, USA

The filamentous fungus *Sordaria macrospora* develops complex fruiting bodies to propagate its sexual spores. Here, we present an analysis of the sterile mutant pro41 that is unable to produce mature fruiting bodies. The mutant is complemented by a small open reading frame encoding the predicted PRO41 protein; the open reading frame and adjacent regions are deleted in mutant pro41. *pro41* transcript levels are upregulated during sexual development. This increase in transcript cannot be observed in the sterile mutant pro1 that lacks a transcription factor, and microarray analysis of gene expression in the mutants pro1, pro41, and the pro1/41 double mutant showed that *pro41* is partly epistatic to *pro1*. PRO41 is predicted to be an ER membrane protein by in silico analyses, and a PRO41- EGFP fusion protein co-localizes with ER-targeted DsRED. Furthermore, Western blot analysis showed that the PRO41- EGFP fusion protein is present in the membrane fraction. A fusion of the predicted N-terminal signal sequence of PRO41 with EGFP is secreted into the medium, indicating that the signal sequence is functional. These data show that PRO41 is a novel ER membrane protein essential for fruiting body formation in filamentous fungi.

363. Characterization of the *Aspergillus nidulans* septin AspA/Cdc11. Rebecca Lindsey, Susan Cowden and Michelle Momany*. Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA lindsey@plantbio.uga.edu

The septin proteins form filamentous rings at the mother-bud neck in yeast. Septins are found in microsporidia, fungi, and animals and are absent from plants. In addition to their original role in cell division, septins have been shown to have roles in cytoskeletal organization, coordination of nuclear division and trafficking across membranes. In *Aspergillus nidulans* there are five septins *aspA/cdc11*, *aspB/cdc3*, *aspC/cdc12*, *aspD/cdc10*, and *aspE*. We have conducted studies on the *A. nidulans* septin *aspA/cdc11*. The null mutant of *aspA/cdc11* is viable and shows uncoordinated germ tubes, hooked

hyphae, split tips, hyperbranching, disorganized conidiophores and hypersensitivity to the actin depolymerizer Cytocholasin A. Localization of AspA/Cdc11-GFP is dynamic. AspA/Cdc11- GFP is visible as a spot on conidia, throughout the cortex in swelling conidia, at the polarizing surface when germ tubes emerge, at septa in germ tubes and branches, and as filaments at tips and in early branches. In *delta aspA/cdc11* SepA-GFP and BimG-GFP localization are lost, but TubA-GFP localization is normal. Our results suggest that AspA/Cdc11 interacts with actin as well as the formin, SepA and the protein phosphatase BimG, possibly in the growing hyphal tip.

364. Characterization of the *Aspergillus nidulans* septin mutant *ΔaspB/cdc3*. Yainitza Rodriguez and Michelle Momany. Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA *yrodriguez@plantbio.uga.edu*

Septins are filament forming P-loop GTPases found in microsporidia, fungi and animals. Septins play important roles in a variety of processes such as cellular and nuclear division, membrane trafficking and organization of the cytoskeleton. This family of proteins was first discovered in a screen for temperature sensitive cell cycle mutants in *Saccharomyces cerevisiae*. In yeast, septins form filamentous rings at the mother-bud neck which are necessary for completion of cytokinesis and normal morphogenesis. In *Aspergillus nidulans* there are five septins *aspA/cdc11*, *aspB/cdc3*, *aspC/cdc12*, *aspD/cdc10*, and *aspE*. The *A. nidulans* septin *aspB/cdc3* was previously shown to localize at septation and branching sites and at interface layers of the conidiophore. This gene was reported to be essential. We have now found that deletion of *aspB/cdc3* is not lethal, but causes severe defects in asexual reproduction and aberrant morphology in several developmental stages. The mutant shows emergence of multiple germ tubes, hyperbranching and hooked branches and disorganized conidiophores. *ΔaspB/cdc3* also shows sensitivity to calcoflour, but not benomyl or cytochalasin A, which suggests that the mutant phenotype is not directly associated with the actin or microtubule cytoskeleton, but perhaps with cell wall stability.

365. Transcriptional changes during the isotropic to polar growth switch in *Aspergillus nidulans*. Andrew Breakspear and Michelle Momany. Department of Plant biology, University of Georgia, Athens, Georgia 30602 USA *abreakspear@plantbio.uga.edu*

Isotropic and polar modes of growth are common to both yeast and filamentous fungi and are fundamental in defining cell shape. Following the breaking of dormancy *A. nidulans* conidia grow isotropically as cell wall components are added uniformly in all directions. After the first nuclear division a switch occurs to polar growth that results in the emergence of a nascent germ tube. The new *A. nidulans* microarray chips produced by the Pathogen Functional Genomics Resource Center (PFGRC) have facilitated the identification of transcriptional changes associated with the switch in growth modes. Microarray analysis was used to compare gene expression before and after germ tube emergence. Following emergence of the primary germ tube 181 genes were down-regulated by at least 2-fold and 401 genes were up- regulated by at least 2-fold. Among up-regulated genes 53 showed a 5-fold or more increase in transcript levels. The results demonstrate that many genes show significant expression changes during early growth in *A. nidulans*.

366. Growth and developmental control in *Aspergillus fumigatus*. Jae-Hyung Mah and Jae-Hyuk Yu. Department of Bacteriology, University of Wisconsin, Madison, WI 53706

The opportunistic human pathogen *Aspergillus fumigatus* reproduces by forming a large number of asexual spores. We studied the mechanisms regulating asexual development in A. fumigatus via examining functions of four key controllers, GpaA (G alpha), AfFlbA (RGS), AfFluG and AfBrlA. Expression analyses of gpaA, AfflbA, AffluG, AfbrlA and AfwetA revealed that, whereas transcripts of AfflbA and AffluG accumulate constantly, AfbrlA and AfwetA are specifically expressed during conidiation. Both loss of function AfflbA and dominant activating GpaAQ204L mutations resulted in reduced conidiation coupled with increased hyphal mass, indicating that GpaA mediates signaling that activates vegetative growth while inhibiting conidiation. As GpaA is the principal target for AfFlbA, the dominant interfering GpaAG203R mutation suppressed the phenotype resulting from loss of AfflbA function. These results corroborate the idea that parimary roles of G proteins and RGSs are conserved in aspergilli. Functions of the two major developmental activators AfFluG and AfBrlA are then examined. While deletion of AfbrlA eliminated conidiation completely, deletion of AffluG did not cause severe defects in A. fumigatus sporulation in air-exposed culture, implying that, whereas the two Aspergillus species may have a common key downstream developmental activator, upstream mechanisms activating brlA may be distinct. Finally, both AffluG and AfflbA mutants showed reduced conidiation and delayed accumulation of AfbrlA mRNA in developmental induction, indicating that these upstream regulators are associated with the proper progression of conidiation.

367. Pdc1, a *Ustilago maydis* 14-3-3 Homologue, Regulates Cell Growth and Separation. Cau D. Pham, Zhanyang Yu, and Michael H. Perlin. University of Louisville, Department of Biology, Program on Disease Evolution, Louisville, Kentucky, USA

14-3-3 proteins are a group of conserved eukaryotic proteins that participate in the regulation of many important cellular processes, e.g., signal transduction, cell cycle regulation, malignant transformation, stress response, and apoptosis. Most organisms have multiple forms of these proteins to provide redundancy or with each isoform fulfilling specific roles. Inspection of the recently-available *U. maydis* genome sequence reveals that this organism may be ideal for the study of 14-3-3 proteins since, in contrast to other organisms, *U. maydis* has only one predicted 14-3-3 protein. We named the *U. maydis* 14-3-3 protein, Pdc1, for its expected role as a phosphorylation-domain coupling protein. Pdc1 appears required for normal cell growth. Haploid strains do no tolerate *pdc1* gene disruption, while Pdc1 knock-down leads to 10-fold reduction of cell density in liquid culture, as well as filamentation and elongation of cells. Calcofluor and DAPI stains show that such elongated or filamentous cells contain multiple septa and nuclei, possibly due to failure of mother and daughter cells to separate. Finally, yeast-2-hybrid data indicate that the Pdc1 protein interacts with a number of proteins (Actin, FPR1 and FBR2, AFG3). Interestingly, Pdc1 interacts with the Rho1 homologue, Uro1, another protein required for cell growth and division, as well as the formin, Bni1, whose interaction with Rho1 in *S. cerevisiae* is required to recruit Ste5 to its G protein. Not unexpectedly, Pdc1 interacts with itself in yeast 2-hybrid experiments. Pdc1 protein can suppress *bmh1bmh2* deletion defects in *S. cerevisiae*, suggesting its functional homology with the 14-3-3 proteins, Bmh1 and Bmh2. Site-directed mutagenesis of the putative protein kinase A target residue in Pdc1 does not prevent dimer formation, but does interfere with the ability of the corresponding mutant Pdc1 proteins to complement *bmh1bmh2 S. cerevisiae* mutant growth defects.

368. Identifying cis- and trans-factors involved in spore-specific gene expression in the oomycete *Phytophthora infestans.* Qijun Xiang, Audrey Ah Fong, Kyoung Su Kim, Howard S. Judelson. Department of Plant Pathology, University of California, Riverside, CA 92521

Using microarray analysis, we have identified a large number of genes induced during sporulation and zoospore formation in *Phytophthora infestans*, the cause of the potato late blight disease. The promoters of several of these genes are being analyzed to define the sequences that confer sporulation or zoospore-specific patterns of gene expression, using a GUS reporter system to test deleted or mutagenized versions of the promoters in *P. infestans* transformants. This approach has been used to identify a 7-nt "cold box" in the promoters of several genes induced during zoospore formation, and to define the binding sites of development-associated transcription factors in the promoters of other genes such as those encoding a sporulation-specific serine-threonine kinase and a protein phosphatase. DNA fragments from the promoters are being used to purify the relevant transcription factors using DNA affinity approaches. EMSA (electrophoretic mobility shift assay) is being used to examine the DNA binding activities of the proteins.

369. A pheromone receptor is involved in mound expression in *Schizophyllum commune*. Goutami Banerjee and Dr. Thomas J Leonard. Clark University, Worcester, MA.

Restriction enzyme-mediated DNA integration (REMI) was used to induce mutations in a homokaryotic strain of *Schizophyllum commune*, Hk28, which forms prolific numbers of large, abnormal multicellular growths called mounds. Mutant transformants were screened for their inability to form mounds. One such moundless transformant was isolated, analyzed and found to have a single disrupted gene caused by plasmid integration. When the disrupted gene was isolated and sequenced, it proved to be an unreported pheromone receptor in Schizophyllum. This transformant was analyzed for its ability to express the hydrophobin genes Sc1, Sc3 and Sc4. Hydrophobin Sc3 is essential for the formation of aerial hyphae in homokaryons and dikaryons, whereas Sc1 and Sc4 are dikaryon specific and are associated with the formation of fruiting bodies. Using Real time PCR, the parent strain HK28 was found to show normal levels of Sc3 gene expression, barely detectable levels of Sc1, and very high levels of Sc4. The moundless transformant was found to express normal levels of Sc3, but showed a sharp decrease and eventual non-expression of hydrophobin Sc4 gene expression during the time period in which mounds are normally produced. These results suggest homokaryotic mound formation and Sc4 gene expression are regulated by a pheromone receptor. When homokaryotic fruiting body tissue was examined for hydrophobin expression, Sc4 was not detected, suggesting the nature of hyphal cohesion involved in homokaryotic fruiting and mound development utilize different hydrophobins for hyphal cohesion.

370. Secondary metabolism and sporulation signalling in *Aspergillus nidulans*. Olivia Márquez[1], Angel Trigos[1], J. Luis Ramos[2], Gustavo Viniegra[3], Holger Deising[4], Nallely Cano[2] and Jesús Aguirre[2]*. [1]Universidad Veracruzana, México. [2]Universidad Nacional Autónoma de México, México. [3]Universidad Autónoma Metropolitana, México. [4]Martin-Luther University Halle-Wittenberg, Germany. *E-mail: jaguirre@ifc.unam.mx

We characterized *Aspergillus nidulans* strains carrying conditional (*cfwA2*) and null (*DcfwA*) mutant alleles of the *cfwA/npgA* gene, encoding an essential phosphopantetheinyl transferase (PPTase). We identified the polyketides shamixanthone, emericellin and dehydroaustinol, as well as the sterols ergosterol, peroxiergosterol and cerevisterol in extracts from developmental cultures. The PPTase CfwA/NpgA was required for production of polyketide compounds, but dispensable for fatty acid biosynthesis. The asexual sporulation defects of *cfwA*, *DfluG* and *DtmpA* mutants were not

rescued by the *cfwA*-dependent compounds identified here. However, *cfwA2* mutation drastically enhanced the sporulation defects of both *DtmpA* and *DfluG* single mutants, suggesting that unidentified CfwA-dependent PKS and/or NRPS are involved in production of unknown compounds, required for sporulation. In addition, we show that *tmpA* and *tmpB* genes define two new *fluG*-independent sporulation pathways.

371. Withdrawn

372. Deletion of the gene encoding the metacaspase homolog PaMCA1 results in lifespan extension in *P. anserina* demonstrating a connection between apoptosis and lifespan control. Andrea Hamann, Diana Brust, Heinz D. Osiewacz Institute of Molecular Biosciences, J. W. Goethe University, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany Phone: + 49 (0)6979829279 Fax: + 49 (0)6979829363 E-mail: a.hamann@bio.uni-frankfurt.de

The wild-type strain s of the filamentous ascomycete *Podospora anserina* is characterized by a mean lifespan of 25 days. The well-characterised senescence syndrome of wild-type strains is accompanied by gross rearrangements of the mitochondrial DNA due to the accumulation of a small circular element, the so-called plDNA finally leading to mitochondrial deficiency. Since mitochondria also play a key role in controlling apoptotic processes, the involvement of an apoptosis machinery in the mechanism by which senescent cultures finally die, was analysed. The genome of *P. anserina* encodes several important apoptosis factors, e.g. two metacaspases and several AIF (apoptosis-inducing factor) and AMID (AIF-homologous mitochondrial inducer of death) homologs. Their putative role in lifespan control was investigated by deleting some of the corresponding genes. Especially the deletion of *PaMca1* results in a 2.5 fold increase in lifespan demonstrating that apoptosis factors are involved in lifespan control. It appears that the strongly elevated level of hydrogen peroxide in senescent mycelium activates a metacaspase-dependent cell death program. This hypothesis is confirmed by the observed enhancement of caspase activity after hydrogen peroxide treatment. Accordingly, during aging the sensitivity of wild-type protoplasts to hydrogen peroxide treatment increases, while it remains stable in a *PaMca1::ble* strain explaining the extended lifespan of this strain. The results obtained in *P. anserina* point to a connection between conserved mechanisms of apoptosis-like processes and organismic aging.

373. The novel regulator of spore maturation VosA controls development and trehalose synthesis in *Aspergillus nidulans.* Min Ni and Jae-Hyuk Yu, Departments of Genetics and Bacteriology, 1925 Willow Drive, University of Wisconsin, Madison, WI 53706, USA

The asexual spore (conidia) is the primary agent for dispersal, survival and propagation in fungi. *Aspergillus nidulans* has served as an excellent model system for studying the mechanism of asexual development. In this report, we identified and characterized the novel developmental regulator VosA that represses asexual/sexual development when present in multiple copies. The VosA (viability of spores) protein is highly conserved in many filamentous fungi, and is similar to VeA, an important regulator of balanced development. As VosA negatively controls conidiation, deletion of vosA resulted in conidiophore development in liquid submerged culture and over-expression of vosA blocked conidiation completely. The regulatory role of VosA in conidiation is primarily via repressing the expression of *brlA*, a key activator conidiation in *Aspergillus*. Importantly, the conidia and sexual spores (ascospores) of the vosA deletion mutant lose cytoplasmic fluids upon prolonged incubation, which results in extremely low (2%) viability of the spores. Chemical analyses revealed that trehalose, a crucial disaccharide serving as a stress protectant, is absent in the conidia of the deletion vosA mutant. These findings coupled with the observation that *vosA* is highly expressed only in conidia and ascospores indicate that VosA plays an essential role in controlling spore maturation and proper regulation of development. In addition, yeast one hybrid experiments indicate that the C-terminal half of VosA may contain a transcriptional activation domain.

374. Functional study of *SMR1/MAT1-1-2*, a mating-type gene which does not control self-non self recognition in *Podospora anserina*. E. Coppin, S. Arnaise, K. Bouhouche, X. Robellet, D. Zickler and R. Debuchy. Institut de Génétique et Microbiologie, UMR 8621 CNRS-Universite Paris Sud, Orsay, France

The sexual reproduction of *P. anserina* requires coordinate action of 4 mating-type genes. Three encode transcriptional factors which control the recognition between *mat- /MAT1-1* and *mat+/MAT1-2* 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/MAT1-1-2*, is essential for development of dikaryotic hyphae; its inactivation results in barren perithecia. In contrast, it is dispensable for development of abnormal uniparental ascogenous hyphae, observed when the other mat genes contain mutations affecting nuclear pairing [1]. *MAT1-1-2* is present in all Sordariomycetes analyzed to date. All MAT1-1-2 proteins contain a 17 aa stretch with conserved and invariant residues forming a HPG domain proposed to define a new DNA- binding domain [2]. However, the cytosolic

localization of the SMR1-GFP protein does not support the hypothesis that SMRl could be a transcription factor. To better understand the structure-function relationships of SMR1 we analyzed 35 alleles constructed by site-directed mutagenesis. Deletion of the HPG domain led to a mutant phenotype, confirming its role in SMR1 activity. However, while 12 aa of this domain were replaced with alanine, only the W193A change inactivated SMR1. We identified a second mutant allele (A347D) in the C terminus, indicating that this region, not conserved in orthologs, is functionally important for sexual development of *P. anserina*. To date the molecular function of SMR1/MAT1-1-2 remains unknown. 1- Arnaise et al., Genetics (2001) 159: 545 2- Debuchy et al., Mol Gen Genet (1993) 241: 667

375. Is RIP abolished at the mating type locus of *Podospora anserina*? K. Bouhouche, E. Coppin and R. Debuchy. Institut de Génétique et Microbiologie, UMR 8621 CNRS-Université Paris Sud, Orsay, France

A mild version of the Repeat Induced Point mutation (RIP), a homology-dependent gene-silencing mechanism, was shown to affect both cis- and trans-duplicated sequences in *P. anserina* (1 and unpublished data). Surprizingly, silencing was never observed for duplications of the *SMR2/MAT1-1-3* mating type gene created at the resident *mat- /MAT1-1* locus by gene replacement, while the same duplication introduced ectopically was silenced by RIP mutations. These data were obtained using a powerful genetic system which permits screening of mutations inactivating *SMR2*, by restoration of sporulation. To extend these results, we introduced a duplication of the *hph* gene conferring hygromycin resistance at the same site of the resident *mat-* idiomorph. As a control, the *mat-* sequence with the duplicated *hph* insertion has been integrated at different ectopic sites. By loss-comparison of *hph* expression in the progeny of crosses involving the different transgenic strains we will be able to determine whether the *mat-* idiomorph is really protected against RIP. This situation, if confirmed, would open a new field of investigation in understanding why and how RIP is specifically abolished at the *mat-* locus (and perhaps at the *mat+* locus). In addition, a genetic system based on *mat* genes expression is already available to seek mutations able to restore RIP at the *mat-* locus after mutagenesis. 1- Graïa et al, Mol. Microbiol. (2001) 40: 586

376. Role of PaTrx1, Patrx2 and PaTrx3, three cytosolic thioredoxins, in the life cycle of the filamentous ascomycete *Podospora anserina.* Fabienne Malagnac[1,2], Benjamin Klapholz[1] and Philippe Silar[1,2]. [1]Institut de Génétique et Microbiologie, UMR CNRS - Université de Paris 11, UPS Bât. 400, 91405 Orsay cedex, France [2]UFR de Biochimie, Université de Paris 7 - Denis Diderot, case 7006, 2 place Jussieu, 75005, Paris, France

Genes encoding thioredoxins were found by searching the complete genome sequence of the filamentous ascomycete *Podospora anserina*. Among them, PaTrx1, PaTrx2 and PaTrx3 are predicted to be canonical cytosolic proteins without additional domains. Targeted disruption of *PaTrx1*, *PaTrx2* and *PaTrx3* showed that PaTrx1 is the major thioredoxin involved in methionine biosynthesis. Deletions also show that either PaTrx1 or PaTrx3 is necessary for sexual reproduction and for the development of the Crippled Growth cell degeneration, processes that are also controlled by the PaMpk1 MAP kinase pathway. Analysis of the phosphorylation and the nuclear localization of PaMpk1 shows that thioredoxins do not participate in the activation of this MAP kinase and thus acts through another pathway.

377. The gene for the ubiquitin ligase regulator Cand1 is putatively split into two genes in the filamentous fungus *Aspergillus nidulans*. Elke U. Schwier, Martin Christmann, Krystyna Nahlik, Silke Busch and Gerhard H. Braus. Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstrasse 8, 37077 Göttingen, Germany; e-mail: eschwie@gwdg.de

Ubiquitin dependent proteolysis plays an important role in many cellular processes in eukaryotes. Cullin containing ubiquitin ligases like the SCF (Skp1-Cullin-F-box protein) complex mark proteins for degradation by ubiquitinylation. It has been shown that the protein Cand1 binds to cullins. The Cand1 C-terminus blocks the SKP1 binding site of the SCF complex component Cul1 and affects thereby the assembly/disassembly of the ubiquitin ligase. The N-terminus of Cand1 buries the neddylation site on Cul1, which prevents its modification by Nedd8, an ubiquitin-like protein and alters the activity of the complex. In *A. nidulans* the gene encoding the putative homolog of human Cand1 seems to be split in two, both independent genes having about 20% identity to the human protein. Deletion of the *A. nidulans* gene coding for the protein Cand1_C similar to the c-terminal part of human Cand1 leads to a red hyphae phenotype. Cand1_C is expressed during vegetative growth but not during sexual development. It localizes to the nucleus and interacts with CulA, CulD and Cand1_N in the yeast two hybrid system. Currently we are investigating the function of the smaller protein Cand_N. The split *cand1* gene makes the fungus *A. nidulans* an attractive model organism for studying the putative different functions of the two parts of the Cand1 protein.

378. B-regulated development is influenced by *sts1*, a putative sugar transporter in the basidiomycete *Schizophyllum commune*. Dionna Kasper, Michael Gallagher, Thomas Hickernell, and Stephen Horton. Department of Biological Sciences, Union College, Schenectady, NY 12308 USA

Sexual development in the basidiomycete mushroom *Schizophyllum commune* is dependent upon activation of signaling pathways controlled by the *A* and *B* mating-type genes. This activation normally occurs as a result of a mating interaction between two compatible haploid individuals, yielding a dikaryon capable of mushroom development. Activation of the B, but not A-regulated pathway results in a characteristic phenotype called flat. The gene *sts1* encodes a putative sugar transporter from *S. commune*, and has been previously implicated in mushroom development. *sts1* null mutant strains were created by homologous integration of a knockout construct in haploid transformation recipients. Dikaryons homozygous for the *sts1* null mutation displayed a severely attenuated ability to fruit. Unexpectedly, when haploid *sts1* null mutants were outcrossed, a proportion of the null progeny displayed the flat phenotype characteristic of B pathway activation. The same phenomenon was observed in regenerated protoplasts of haploid *sts1* null strains. In both cases, the flat individuals were found to donate, but not accept nuclei in test matings, a genetic behavior consistent with the idea that B-regulated development was indeed activated in these haploid strains. From these data, we hypothesize that *sts1* plays a role in both B-regulated development and fruiting body production in *S. commune*.

379. Cis-acting elements regulating transcription initiation and sporulation-specific expression of the *PiCdc14* gene of *Phytophthora infestans*. Audrey M. V. Ah Fong and Howard S. Judelson. Department of Plant Pathology, University of California, Riverside, California, USA.

Asexual spores are central to the disease cycle of *Phytophthora infestans,* the cause of tomato and potato late blight. To understand transcriptional processes involved in sporulation, functional regions were identified within the promoter of *PiCdc14,* a gene normally expressed only during asexual spore development. These were defined by testing truncated promoters or site-directed mutants in transformants of *P. infestans,* using the GUS reporter. Two functional classes of motifs were identified, one directing sporulation-specific expression and others regulating the position of transcription initiation. Two apparently redundant binding sites for transcription factors directing expression during sporulation were localized just upstream of nt -66 (relative to transcription start site), within a region showing high conservation in four *Phytophthora* spp. The site of transcription initiation was controlled both by sequences flanking the normal major start site and the motif CTCAAC, which was present four times between -74 and -138. Promoters in which either the normal start site or all four CTCAAC motifs were eliminated still exhibited sporulation- specific expression, but initiation commonly occurred at nt -109. Deletions of single CTCAAC motifs had intermediate effects. Although the region containing the major start site of *PiCdc14* lacks the Inr-like consensus described previously for many other *Phytophthora* genes, it does not appear to represent a sporulation-related determinant since exchanging it with the Inr-like region of the *PiExo1* gene did not impair *PiCdc14* expression. Experiments to better define the biochemical and genetic interactions between these sites and their cognate transcription factors are underway.

380. *GzPP2A*, encoding a putative protein phosphatase 2A, is involved in sexual development and pathogenesis in *Gibberella zeae.* Hee-Kyoung Kim[1], Yin-Won Lee[2], and Sung-Hwan Yun[1]. [1]Department of Biological Resources and Technology, Soonchunhyang University, Asan 336-745; [2]School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea.

***Gibberella zeae* is an important pathogen of maize, wheat and rice. We used REMI to identify a self-sterile mutant of *G. zeae*, designated R2629. The REMI mutant showed pleiotropic phenotypes other than no perithecial formation, including abnormal conidia morphology and reduction in mycelial growth and virulence on host plants. Outcrossing analysis confirmed that all of the phenotypic changes in R2629 were tagged with the hygromycin B resistance marker. The vector insertion appears to cause a complicated genomic rearrangement in R2629. The genomic region flanking 5' of vector insertion site was identified as the *Kpn*I site 713 bp upstream of the ORF annotated as FG01141.1 located in the contig 1.56 of the *G. zeae* genome databases, which showed a similarity to delta-1- pyrroline-5-carboxylate dehydrogenase, whereas the 3' flanking region was matched to a *Kpn*I site within the ORF designated *GzPP2A* (FG05894.1 located in the contig 1.236) highly similar to B56-delta regulatory subunit of protein phosphatase 2A. Subsequent targeted gene deletion and complementation analyses confirmed that *GzPP2A* was responsible for the pleiotropic phenotypic changes in the original REMI mutant. The GFP gene under the control of the *GzPP2A* promoter was strongly expressed in both mycelia and conidia, but not in ascospores, indicating that *GzPP2A* is down-regulated during the sexual reproduction mode. Taken together, it is conclusive that *GzPP2A* regulates important cellular processes in *G. zeae*, including fungal development (during both asexual and sexual stages) and pathogenicity on host plants.**

381. Identification of proteins interacting with the *Sordaria macrospora* WD40 repeat protein PRO11 by a yeast two-hybrid screen. Yasmine Bernhards and Stefanie Pöggeler. Georg-August-University of Göttingen, Institute of Microbiology and Genetics, Department Genetics of eukaryotic microorganisms, Göttingen, Germany.

Fruiting-body development in fungi is a complex cellular differentiation process that is controlled by more than 100 developmental genes. Mutants of the filamentous ascomycete *Sordaria macrospora* showing defects in fruiting- body formation are pertinent sources for the identification of components of this multicellular differentiation process. Recently,

we have shown that the sterile mutant pro11 carries a defect in the *pro11* gene encoding a multimodular WD40 repeat protein. PRO11 shows significant homology to several vertebrate WD40 proteins, such as striatin and zinedin, which are supposed to function as scaffolding proteins linking signaling and eukaryotic endocytosis. In this study, a yeast two-hybrid screen of a *S. macrospora* cDNA library with the *pro11* cDNA has been used to identify interaction partners of PRO11. With PRO11 as a bait, amongst others we discovered phocein, a putative homologue of the mammalian phocein as putative interaction partner. In mammals, phocein was discovered by a yeast two-hybrid screen using striatin as a bait. Phocein bears a few homologies with the sigma-subunits of clathrin adaptor proteins. Using phocein as a bait in a yeast two-hybrid screen, we identified novel interacting proteins. Amongst the proteins of interest are subunits of the COPI vesicle coat and subunits of the 20S proteasome.

382. Networks of *Sordaria macrospora* transcription factors: interactions between the MADS box protein MCM1, mating type proteins and the homeodomain transcription factor STE12. Nicole Nolting* and Stefanie Pöggeler. Department of Genetics of Eukaryotic Microorganisms - Institute of Microbiology and Genetics –Georg-August University Göttingen, Germany

The MADS box protein MCM1 controls diverse developmental processes and is essential for fruiting body formation in the homothallic ascomycete *Sordaria macrospora*. In *S. macrospora*, deletion of *mcm1* results in a pleiotropic phenotype. *S. macrospora* deltamcm1 strains display a reduced biomass, increased hyphal branching and reduced hyphal compartment length during vegetative growth. Furthermore, they are unable to produce fruiting bodies during sexual development. Little is known about interaction partners of MADS box proteins in filamentous fungi. In *S. macrospora*, MCM1 interacts with the mating-type protein SMTA-1. In order to identify more interaction partners, we have screened a *S. macrospora* cDNA library. From this screen, the *S. macrospora ste12* gene, a homologue of *Saccharomyces cerevisiae* STE12, was identified. In *S. cerevisiae*, STE12 is responsible for activating genes in response to MAP kinase cascades controlling mating and filamentous growth. The *ste12* gene of *S. macrospora* encodes a protein containing both, a homeodomain and a zinc finger region. Deletion of the *ste12* gene in *S. macrospora* neither affects vegetative growth nor fruiting body formation. However, ascus and ascospore development are highly impaired by the deltaste12 mutation. Our data provide an example for the functional divergence of STE12 homologues in filamentous ascomycetes.

383. The *Aspergillus nidulans* F-box Protein Project Özgür Bayram[1], Heike S. Rupprecht[1], Marc Dumkow[1], Marcia R. v. Z. Kress[1], Thomas Linger[2], Özlem Sarikaya Bayram[1], Gustavo H. Goldman[3], Gerhard H. Braus[1]. [1]Institute of Microbiology & Genetics, Georg-August-University Goettingen, Grisebachstr. 8, 37077 Goettingen, Germany; e-mail: obayram@gwdg.de; hruppre@gwdg.de [2]Institute of Microbiology & Genetics, Dept. of Bioinformatics, Georg-August-University Goettingen, Goldschmidtstr. 1, 37077 Goettingen, Germany [3]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, CEP 14040-903, Ribeirão Preto, São Paulo, Brazil

F-box proteins are part of E3 SCF (Skp1,Cullin,F-box) ubiquitin ligases which are the specificity factors of the ubiquitin dependent protein degradation machinery. They contain the F-box as conserved N-terminal domain which acts as interface to the Skp1 protein. In addition they have a substrate binding domain for the protein to be ubiquitinated. There are at least 70 F-box-like proteins encoded by the *Aspergillus nidulans* genome. To address the cellular functions of F-box proteins in *A. nidulans*, we have started to systematically disrupt F-box encoding genes via homologous gene replacement method. The characterization of the F-box mutants, which has been carried out so far, will be presented.

384. Mutations in mating-type genes greatly decrease repeat-induced point mutation in the fungus *Podospora anserina*. Sylvie Arnaise, Denise Zickler, Anne Bourdais and Robert Debuchy. Institut de Genetique et Microbiologie, Bat 400, Univ Paris-Sud, UMR 8621, Orsay, F-91405.

RIP (Repeat-Induced Point mutation) and PR (Premeiotic Recombination) are two developmentally regulated silencing processes in filamentous ascomycetes. RIP detects and mutates duplicated sequences, while PR results in deletion of the interstitial sequence between cis-duplicated sequences. These processes take place in the fruiting bodies, between fertilization and premeiotic replication. Fertilization is controlled by the mating types, which have been demonstrated to control also subsequent developmental events preceding meiosis in *Podospora anserina* and *Neurospora crassa*. We have investigated the relationship between mating type and silencing processes in *P. anserina*. We thus tested several mutations in the mating-type genes which affect fruiting-body development, for their effects on RIP and PR. These mutations strongly decrease the frequency of the silencing processes, a clear indication that the two processes are connected. Possible models for the relation between the mating-type genes and the silencing processes will be presented.

385. A mutation in a GDP mannose pyrophosphorylase encoding gene leads to aberrant hyphal growth. *Brian D. Shaw, Gustavo Rebello, Soo Chan Lee, Srijana Upadhyay and Melissa Long. Program for the Biology of Filamentous Fungi, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77803, USA.

The temperature sensitive *swoN1* mutant results in an aberrant hyphal growth pattern that differs significantly from wild type. Growth from conidia results in germlings with pronounced swollen sub-apical cell compartments as much as 20 micron in diameter. Growth and development at hyphal apices appears to proceed similarly to wild type. The *swoN1* mutant was complemented using a genomic library, in which two genes were identified with potential to restore growth of the mutant to wild type levels. These genes are AN1911.3 and AN5586.3 as designated by the Broad Institute *A. nidulans* genomic database; each encode proteins with predicted similarity to GDP mannose pyrophosphorylase. We designate AN1911.3 *swoN* since it contains a point mutation at position 1240 bp after start of genomic sequence and 1093 bp after the start codon of coding sequence. This point mutation results in a predicted residue change of serine to a phenylalanine at amino acid 365 of the protein. The *swoN1* mutant also exhibits an altered staining pattern of the mannoprotein stain Alcian Blue, relative to wild type. This gene along with *swoM, manA* and *swoA* represents the fourth identified in *A. nidulans* that is likely to participate in protein mannosylation. Each of these mutations results in swollen cells with aberrant hyphal growth, suggesting that cell wall associated mannoproteins are important for maintaining hyphal shape.

386. The role of ADP-ribosylation factors in cell morphogenesis of *Aspergillus nidulans.* Soo Chan Lee and Brian D. Shaw. Program for the Biology of Filamentous Fungi/Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, 77840

ADP-ribosylation factors (ARFs) are small GTPase proteins with several biological activities including vesicle formation and trafficking and, in yeast, bud site selection. In filamentous fungi, numerous vesicles are found at the growing tips and in the Spitzenkorper where they are thought to be active in secretion of cellular components, endocytosis, and maintenance of tip growth. The exact roles of the ARFs in filamentous fungi have not been established. ArfA::GFP localizes to cellular compartment which may be Golgi. ArfA::GFP was not co-localized with endocytosis machineries indicating ArfA is involved in exocytosis. ArfB::GFP localized to septa, a new cell wall synthesis site. Disruption of ArfB by transposon insertion resulted in loss of polarity during germ tube emergence and hyphal growth. A compromised Spitzenkorper was observed in the mutant. In addition, the arfB::Tn strain displayed delayed endocytosis. The Arf proteins have a conserved N-myristoylation motif. In *swoF1* (N-myristoyl transferase) mutant cells, ArfA::GFP and ArfB::GFP showed non-specific localization. In wild type cells, ArfA^{G2A}::GFP and ArfB^{G2A}::GFP, each with a G2A amino acid substitution that disrupts myristoylation, mislocalized. Interestingly overexpression of ArfA protein partially rescues the polarity defect of the *swoF1* mutant. These observations suggest that both endocytosis and exocytosis by the Arf proteins play a critical role in hyphal polarized growth in filamentous fungi and N-myristoylation determines subcellular localizations for ArfA and ArfB.

387. A link between N-myristoylation and proteasome activity. Soo Chan Lee and Brian D. Shaw. Program for the Biology of Filamentous Fungi/Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, 77840

Ubiquitin dependent proteolysis is a fundamental biological process regulating the half-life of proteins. The interaction between the 19S and 20S proteasome particles is essential for this activity. A subunit of the 19S particle, RptA in *Aspergillus nidulans*, has a conserved N-myristoylation motif. Orthologs of RptA are known to control substrate entry and gate the channel of 20S particle, but less is known about the role of N-myristoylation of the protein on its function. In our analyses of genetic suppressors of an *A. nidulans* N-myristoyl transferase mutant (*swoF1*), we found a mutation in a 20S proteasome alpha subunit that partially bypasses phenotypic defects of the *swoF1* mutant. To investigate the mode of suppression, we used monoclonal anti-ubiquitin antibody to measure the amount of the ubiquitinated proteins. The *swoF1* mutant accumulates fewer ubiquitinated proteins than does wild-type. The suppressor mutant, however, accumulated more. In addition, the abnormal hyphal growth phenotypes of the *swoF1* mutant were partially bypassed in the presence of MG132, a proteasome inhibitor. These data suggest that N- myristoylation negatively regulates the proteasome activity. We will discuss our ongoing investigation of the role of myristoylation of RptA protein on i) function of the proteasome, ii) the interaction between 19S and 20S proteasome and iii) the localization of 19S proteasome.

388. PRM-1 is involved in germling fusion and is essential for male and female fertility in *Neurospora crassa.* Andre Fleissner, Spencer Diamond and N. Louise Glass. Department of Plant and Microbial Biology, University of California, Berkeley, USA

Germ tubes of *N. crassa* conidia show mutual attraction and fusion resulting in the connection of many individuals to one functional structure. In *Saccharomyces cerevisiae* Prm1p is essential for efficient plasma membrane fusion between mating cells. The *N. crassa* genome contains a *PRM-1* homolog. To study the potential role of the respective protein in germling fusion, we constructed a *prm-1* knock out mutant in *N. crassa*. Germ tubes of germinating *prm-1* conidia show normal attraction and make contact similar to the wild type. Quantitative fusion analysis showed that more than 50% of mutant germling pairs remain unfused, suggesting a role of PRM-1 in membrane fusion. Additionally *prm-1* mutants exhibit a dominant phenotype during sexual development. The mutant shows normal protoperithecia development but is

sterile both as the male or female mating partner in crosses with wild type. Perithecia show initial normal development after fertilization, such as enlargement and darkening, suggesting that fusion between the mating partners is normal. However, further development is arrested and perithecia are devoid of progeny. These data indicate a novel essential role of PRM-1 in post-fertilization events.

389. Characterization of genes expressed during sexual reproduction in *Phytophthora.* Xiaofan Niu, Waraporn Prakob, and Howard S. Judelson. Department of Plant Pathology, University of California, Riverside CA 92521 USA.

Sexual reproduction serves an important role in the life and disease cycles of *Phytophthora* since the sexual spores (oospores) result in new genotypes and are an important means of long-term survival. In order to understand the mechanism of sexual development in the genus, *P. infestans*, the causal agent of potato and tomato late blight, is being used as a model in our study. Microarrays representing much of the *P. infestans* transcriptome were used to profile gene expression during mating. Approximately 87 genes induced more than ten-fold were identified using these arrays and validated by semi-quantitative RT-PCR. A majority of these genes are induced exclusively during mating, but some are also up-regulated during asexual spore development. Most are expressed during oosporogenesis in the homothallic species *P. phaseoli.* To understand the temporal and spatial patterns of expression of the genes, the activity of ten genes transcribed only during mating are being tested in *P. infestans* transformants by fusing their promoters with the GUS reporter gene. Deletions, site-directed mutagenesis, and electrophoresis mobility gel shift assays will be used to define the *cis*-elements regulating the genes. In addition, the function of selected mating-induced proteins are being tested, such as a RNA-binding protein belonging to the Puf family. Affinity strategies are identifying mRNAs bound and presumably regulated by Puf.

390. Investigations on protein interactions between HET-C and PIN-C during heterokaryon incompatibility in *Neurospora crassa*. Jianping Sun, Julie Welch and N. Louise Glass. Plant and Microbial Biology Department, University of California, Berkeley, California, 94720

Self/nonself discrimination is a ubiquitous and essential function in both multicellular and microbial species. In filamentous fungi, such as *Neurospora crassa*, nonself recognition is important during both sexual reproduction and vegetative growth. During vegetative growth, hyphal fusion between individuals that differ in specificity at *het* loci results in rejection of heterokaryon formation and programmed cell death. Heterokaryon incompatibility (HI) has been shown to reduce the risk of transmission of infectious cytoplasmic elements. In *N. crassa*, *het-c* HI is mediated by nonallelic interactions between the closely linked loci, *het-c* and *pin-c*, suggesting that physical interaction between HET-C and PIN-C is required for nonself recognition. In order to investigate the possible physical interaction between HET-C and PIN-C, localization and affinity purification (LAP) will be used. The *het- c* locus encodes a glycine-rich plasma membrane protein, while *pin-c* encodes a HET domain protein. Population analyses of *het-c/ pin-c* showed that alleles at *het-c* and *pin-c* show severe linkage disequilibrium; *pin-c* alleles are extremely polymorphic and show only ~50% DNA sequence identity. Analysis of the transcriptional start site of *pin-c* alleles by 5' RACE showed alternative start sites. These experiments will provide further information towards understanding the mechanism and evolution nonself recognition in filamentous fungi.

391. Classic sexual reproduction and monokaryotic fruiting are differentially regulated by the mediator protein Ssn8 in *Cryptococcus neoformans*. Yu-Sheng Lin and Wei-Chiang Shen. Department of Plant Pathology and Microbiology, National Taiwan University, Taiwan. wcshen@ntu.edu.tw

***Cryptococcus neoformans* is an important human fungal pathogen with defined life cycle. The well developed molecular and genomic resources have established it as an excellent model for studying fungal virulence and physiology. Our previous study demonstrated that *C. neoformans* can perceive blue light and blue light represses sexual filamentation via the conserved white collar proteins, Cwc1 and Cwc2. To further understand how blue light negatively regulates mating filamentation, we conducted a suppressor screening using *Agrobacterium*-mediated transformation under *CWC1* overexpression strain background. After screening 4132 nourseothricin-resistant transformans, we obtained 64 strains with different levels of filamentation phenotypes. In this presentation, we report the characterization of an *Agrobacterium* transformant AY18 in which T-DNA integration affected the activity of a gene homologous to the *Saccharomyces cerevisiae SSN8*. To reveal the roles of *C. neoformans SSN8* homologue, we created the *ssn8* deletion mutants under different genetic backgrounds. *SSN8* gene deletion in the *CWC1* overexpression strain displayed similar filamentation as the original AY18 T-DNA integrant. Furthermore, the wild type *MATalpha f*nand *MATa ssn8* mutants were obtained, and both mutants displayed the reduction level of filamentation in classic alpha-a cells mating. More interestingly, dramatic production of monokaryotic filamentation, a rare sexual differentiation involved the same sex of mating cells, was observed in the *MATalpha f*nand even in the *MATa ssn8* mutants. Our data suggest that *C. neoformans SSN8* homologue plays critical but divergent roles in two sexual differentiation processes of *C. neoformans*. This is the first demonstration**

of mediator component involved in the differentiation of *C. neoformans*. Its role related to the Cwc complex is under investigation.

392. The *smtA* Gene Encoding a Putative SAM Methyl Transferase is Necessary for Progression of both Sexual and Asexual Development of *Aspergillus nidulans.* Hyo-Jung Kim, Jee-Hyun Kim*, Keon-Sang Chae[1], Kwang-Yeop Jahng[1] and Dong-Min Han. Institute of Basic Natural Science, Wonkwang University, [1] Div. Biological Science, Chonbuk National University

The *smtA*(S-adenoslymethionin Methyl Transferase) gene was identified as a multi-copy suppressor of various mutations such as *sndE80*, *silC188*, *silD6*, *silE181* and *silF174*, which overcame the inhibitory effect of stresses on sexual development. The gene is predicted to encode a putative methyl transferase carrying an S-adenosylmethionin binding domain. The *smtA* ORF consists of 353 amino acids and is disrupted by 9 introns. The null mutant showed defects both in sexual and asexual developments. Conidiation and cleistothecia maturation were delayed and the amounts were reduced. The *smtA* gene was expressed in high level during vegetative growth and maintained only upto the early stages of asexual and sexual development. The *smtA* mRNA level was largely reduced in *veA* or *fluG* null mutant indicating that the *smtA* gene expression was positively controlled by those genes. The *veA* null mutation was epistatic to *smtA* mull mutation. These results together suggest that SmtA play some role in progression of both sexual and asexual development downstream of VeA and FluG.

393. Analysis of *pceA*, a regulatory gene for early stage of conidiation in *Aspergillus oryzae*. Kenichiro Matsushima[1], Osamu Hatamoto[2], Genryou Umitsuki[1] and Yasuji Koyama[1]. [1]Noda Institute for Scientific Research, [2]Product Development Division, Kikkoman Corporation. Japan.

The *pceA* gene of *A. oryzae*, encoding a putative transcription factor with a GAL4-like Zn(II)2Cys6 motif, is an ortholog of the *A. nidulans nosA* gene, which is involved in regulation of sexual development. KN16-10, a *pceA* overexpressing strain of *A. oryzae*, exhibited an earlier and increased conidiation compared to the wild-type strain. In addition, KN16-10 produced conidiospores in liquid medium, whereas the wild-type strain did not produce them under the same conditions. The expression of conidiation-related gene homologs, such as *brlA* and *flbA*, and that of the *pceB*, a paralog of *pceA*, were investigated in KN16-10 and the wild-type strain. The Real-Time PCR analysis showed that expression of *brlA*, a regulatory gene for the early stage of conidiation in *A. nidulans*, increased in KN16-10 both in liquid and solid medium. At the same time, the expression of *pceB* increased in parallel with the expression of *pceA*. These findings suggest that *pceA* plays an important regulatory role in the early stage of conidiation in *A. oryzae* by upregulating the expression of *brlA*. Finally, considering that the expression of *pceB* seems to be also upregulated by *pceA*, *pceB* might be involved in conidiation as well, although its exact function remains unclear.

394. Two G protein alpha subunits share redundant roles in pheromone sensing in *Cryptococcus neoformans.* Yen-Ping Hsueh, Chaoyang Xue and Joseph Heitman. Department of Molecular Genetics and Microbiology, Duke University, Durham, NC

A vast majority of the cell-environment communication is dependent on the G protein-coupled receptors (GPCR) and their cognate G proteins that act as molecular switches for signaling cascades that respond to different cues. In fungi, one such signaling cascade is the well characterized pheromone response pathway triggered by pheromone/pheromone receptor recognition. Unlike *Sacchromyces cerevisiae* which expresses two G alpha subunits (one coupled to the pheromone receptor and the other to the nutrient sensor Gpr1), most filamentous ascomycetes and basidiomycetes have three G alpha subunits. Studies in many fungi have successfully identified and characterized the G alpha subunit that acts upstream of the cAMP-PKA pathway, while it has been unclear which of the G alpha subunits is coupled to the pheromone receptor and pheromone response pathway. Here we report that in *Cryptococcus neoformans* , a pathogenic basidiomycetous yeast, two G protein alpha subunits (Gpa2 and Gpa3) are both involved in pheromone sensing and mating. Genetic analysis reveals that *gpa2 gpa3*double mutants, but not either the *gpa2* or *gpa3* single mutant, are sterile in bilateral cross, indicative of a shared role in the mating process. Deletion of *GPA3* causes constitutively active pheromone response and hyperfilamentation in haploid cells. Furthermore, the expression of *GPA2* and *GPA3* is differentially regulated. *GPA3* expression is induced in the nutrient-limiting condition while *GPA2* is specifically induced during mating. Overexpression of a dominant active *GPA2* allele enhances pheromone expression while overexpression of a dominant active *GPA3* allele results in mating inhibition. This suggests that Gpa2 and Gpa3 have both redundant and divergent signaling activities governing the sexual development. The incorporation of an additional G protein alpha subunit into the regulatory network enables increased signaling complexity and may facilitate cell fate decisions involving the choice between filamentous sexual and asexual development.

395. Photoregulation of apothecial morphogenesis and patterning in *Sclerotinia sclerotiorum.* Jeffrey A. Rollins and Selvakumar Veluchamy. Department of Plant Pathology, University of Florida, Gainesville, FL E-mail: rollinsj@ufl.edu

We have undertaken spectral characterization studies to determine the wavelengths of light that positively influence apothecial photomorphogenesis and phototropisms in *Sclerotinia sclerotiorum* . Our results indicate that wavelengths between 320 and 400 nm (UV-A) are sufficient and necessary for apothecial morphogenesis and positive phototropism. Wavelengths in the blue to green (425-520 nm) range of the spectrum are negatively phototropic at low fluence rates and can de-etiolate stipes at high fluence rates. Insight into the spatial coordination of apothecial development was also investigated by light deprivation experiments. The effect of removing light after the initiation of photomorphogenesis resulted in the abortion of the existent disc development and stage-specific redevelopment of new stipes or discs. To identify the primary photoreceptor(s) governing these photoresponses, we have begun functional characterization of putative photoreceptor-encoding genes identified from the *S. sclerotiorum* genome sequencing project (https://www.broad.mit.edu/annotation/fgi). Based on Northern hybridization analyses, Ss- *cry1* which encodes a member of the CRY-DASH photolyase sub-family, is the first candidate gene targeted for functional analysis. Ss- *cry1* transcripts do not occur in dark germinated stipes, but accumulate by 15 min. and peak around 6 h. in stipes exposed to UV-A (3.5µmoles/m ^{2} /s). No wavelengths in the visible spectrum were found to stimulate significant Ss- *cry1* transcript accumulation. We have isolated Ss- *cry1* gene replacement mutants and are working to genetically purify and phenotypically characterize these mutants.

396. The roles of fimbrin, *fimA*, and alpha-actinin, *acnA*, in hyphal growth. Srijana Upadhyay[1], Aleksandra Virag [2], Soo Chan Lee [1], Steven D. Harris[2], and Brian D. Shaw [1]. [1]Dept of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77803, USA. [2]Plant Science Initiative, University of Nebraska-Lincoln, N234 Beadle Center, 1901 Vine Street, Lincoln, NE 68588, USA.

We investigated the roles of actin (ActA) binding proteins fimbrin (FimA) and alpha-actinin (AcnA) in hyphal growth in *A. nidulans*. We have used live cell imaging to examine the distribution of ActA::GFP, FimA::GFP and AcnA::GFP. In actively growing hyphae cortical ActA::GFP and FimA::GFP patches are highly mobile and are concentrated near the hyphal apex, but a patch depeleted zone occupies the hyphal apex. FimA::GFP localizes transiently to septa. Localization of both ActA::GFP and FimA::GFP was disrupted after cytochalasin treatment. AcnA::GFP localizes to septal rings and has not been visualized at hyphal apices. A transposon insertional strategy was used to disrupt *fimA* resulting in germinating conidia with an extended isotropic growth phase followed by simultaneous emergence of multiple germ tubes. Colonies of the *fimA* disruptants are compact and conidiate poorly. Deletion of *acnA*, results in a severe hyphal growth defect leading to compact colonies that not sporulate. Endocytosis was severly impaired in the *fimA* disruption strain but was unaffected in the *acnA* deletion strain. ActA::GFP distribution in the *fimA* disruption strain, results in abnormal ActA::GFP distribution. A model for the roles of these proteins in hyphal growth is proposed.

397. Involvement of G-alpha subunits of heterotrimeric G proteins and adenylyl cyclase in fruiting body development of *Sordaria macrospora.* Jens Kamerewerd, Malin Jansson, Minou Nowrousian, Stefanie Pöggeler and Ulrich Kück. Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, Universitätsstr. 150, 44780 Bochum, Germany, jens.kamerewerd@rub.de

Heterotrimeric G proteins play an important part in signal transduction and regulation of fungal differentiation and growth. The relative contribution of *Sordaria macrospora* G-alpha subunits GSA1, GSA2 and GSA3 in vegetative and sexual development has been shown through analysis of strains deleted for various combinations of the corresponding genes. All mutants lacking one of the G-alpha subunits are restricted in vegetative growth. Strains lacking the *gsa1* gene in addition to one of the other subunits are completely sterile. Defects caused by the deletion of *gsa2* are observed only in combination with loss of *gsa1*, suggesting that this G-alpha subunit acts complementary to the other subunits in *S. macrospora*. GSA3 seems to be involved in the germination or viability of ascospores. To characterize the possible involvement of adenylyl cyclase as downstream effector, we generated a mutant lacking the *cr1* gene. Besides restricted mycelial growth, the perithecia of the deletion strain accumulate mainly under the surface of solid media. The mutant is fertile, but germination of ascospores is reduced. Analysis of double knockout mutants with G-alpha subunits and adenylyl cyclase suggests a major role of GSA1 and CR1 for perithecial development as the double mutant develops no mature perithecia. The sum of our results indicates that G-alpha subunits and adenylyl cyclase are not essential for viability of this filamentous fungus but are important factors for sexual propagation.

398. The Woronin body-associated PRO40 protein is required for fruiting body development in *Sordaria macrospora.* Ines Engh [1], Christian Würtz[2], Konstanze Witzel-Schlömp [1], Hai Yu Zhang [1], Birgit Hoff [1], Minou Nowrousian [1], Hanspeter Rottensteiner [2] & Ulrich Kück[1]. Lehrstuhl für Allgemeine und Molekulare Botanik [1] und Institut für Physiologische Chemie, Abteilung für Systembiochemie, [2] Ruhr-Universität Bochum, Universitätsstraße 150, 44780 Bochum, Germany, ines.engh@rub.de

Fruiting body formation in ascomycetes is a highly complex process that is under polygenic control. However, the molecular determinants regulating this cellular process are largely unknown. Here we show that the sterile pro40 mutant

of the homothallic ascomycete *Sordaria macrospora* is defective in a 120 kDa WW domain protein that plays a pivotal role in fruiting body maturation. Complementation analysis with different pro40 mutant strains using full-sized or truncated versions of the wild-type *pro40* gene revealed that the C-terminus of PRO40 is crucial for restoring the fertile phenotype. Microscopic investigations using DsRed or GFP polypeptides showed a co-localization of PRO40 with HEX-1, a Woronin body specific protein. However, fluorescence microscopy, sedimentation and immunoblotting analyses as well as growth tests with the *S. macrospora* pro40 mutant and *Neurospora crassa* mutants of the *pro40* homolog *so* revealed that Woronin body integrity is not affected in mutant strains. We discuss the function of PRO40 in fruiting body formation.

399. Pheromone functions of *Gibberella zeae*. Jungkwan Lee [1], Robert L. Bowden [2], and John F. Leslie [1]. [1] Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS66506-5502, [2] USDA-ARS Plant Science and Entomology Research Unit, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS66505-5502.

In heterothallic Ascomycete fungi, mating specificity for sexual reproduction is controlled by two idiomorphs (*MAT- 1* and *MAT-2*). Two sex pheromone/receptor pairs function in recognition and attraction of opposite mating types. In the homothallic fungus *Gibberella zeae* (*Fusarium graminearum*), the *MAT* locus has been rearranged such that both of the idiomorphic alleles are next to one another on the same chromosome. Our objective was to characterize pheromone precursor genes (*ppg1* and *ppg2*) and pheromone receptor genes (*pre1* and *pre2*) from this fungus. *ppg1* is expressed in germinating conidia and mature ascospores of *G. zeae*, but *ppg2* expression was not detected in any cells. *pre2* was expressed in all cells including mycelial cells induced on carrot agar, whereas *pre1* was weakly expressed only in mature ascospores. Deletion of *ppg1* or *pre2* (*Δppg1* or *Δpre2*) reduced the number of perithecia but *Δppg2* and *Δpre1* mutants had no discernable change in morphological phenotype or sexual fertility. A *Δppg1*/*Δppg2* double mutant has a phenotype similar to that of the *Δppg1* single mutant, suggesting that neither pheromone is essential for either male or female fertility. *Δppg1* or *Δppg2* in combination with a partially disabled *MAT* allele did not result in any other changes in either male or female fertility beyond those expected for the *Δppg1* or *Δppg2* mutations alone. We conclude that one of the pheromone/receptor pairs (*ppg1*/*pre2*) found in many Ascomycetes has a role in, but is not essential for, selfing and outcrossing in *G. zeae*, whereas the other pheromone/receptor pair (*ppg2*/*pre1*) no longer has a function in sexual reproduction.

400. Catalases during the sexual cycle of the filamentous fungus *Podospora anserina*. Eric Espagne, Anne Bourdais, Denise Zickler and Veronique Berteaux-Lecellier. Institut de Genetique et Microbiologie, Universite Paris-Sud-11, UMR8621,F-91405 Orsay, France.

Reactive oxygen species are not only toxic molecules but they are also signaling molecules that control various processes such as pathogen defense, programmed cell death and cell differentiation. Our previous studies of the *pex2* peroxisome assembly mutants, which dikaryotic cells never engage into karyogamy and meiosis but remain in a proliferative state, suggested a role of hydrogen peroxide in *P. anserina* differentiation. An increase in hydrogen peroxide level might be responsible for the failure in the meiotic engagement and/or for the anomalous maintenance of the mitotic state. To test this hypothesis and more generally to study the role of hydrogen peroxide during *P. anserina* life cycle, we proposed to increase or reduce the amount of hydrogen peroxide by deregulating genes involved in its production or degradation. In a first attempt, we focused on catalase genes which encode enzymes that convert hydrogen peroxide to oxygen and water. We have identified 4 monofunctional catalases and one bifunctional catalase- peroxidase in the *P. anserina* genome. Expression of the 5 catalases has been followed during the sexual development and 3 of them have been deregulated. Characterization of the strains is under investigation. The results as a whole will be discussed in the frame of the role of hydrogen peroxide in the fungus life cycle.

401. Peroxisomal ABC transporters and beta-oxidation in the filamentous fungus *Podospora anserina*. Stephanie Boisnard, Eric Espagne, Denise Zickler, Anne-Laure Riquet, Anne Bourdais and Veronique Berteaux-Lecellier. Institut de Genetique et Microbiologie, Universite Paris-Sud, UMR8621, F-91405 Orsay, France. The first two authors contributed equally to the work.

The two *P. anserina* peroxisomal ABC transporters pABC1 and pABC2 display respectively 39% and 45% identity with the human ALDP peroxisomal ABC transporter (adrenoleucodystrophy). Although ALDP's function remains unclear, various data suggest that it could be involved in the transport of acyl-CoAs across the peroxisomal membrane where they will be oxidized. We have shown that overproduction of human PMP70 and pABC1 transporters in *P. anserina*, act as partial suppressors of the differentiation defect of the *pex2* peroxisomal assembly mutant (Zellweger syndrome in humans). To determine more precisely the involvement of peroxisomal ABC transporters in both acyl-CoA transport across the peroxisomal membrane and their possible role in differentiation, we knocked out both *pABC1* and *pABC2* genes. Contrary to their counterpart in budding yeast, the single and double deleted strains do not display growth deficiency on oleic acid as sole carbon source. The further discovery of a mitochondrial beta-oxidation in filamentous

fungi suggested that this pathway might compensate for the lack of peroxisomal oleic acid oxidation in the *delpABC1 delpABC2* double mutant and lead us to characterize mutants affected in both mitochondrial and peroxisomal beta-oxidation pathways.

402. Identification of two genes in *Gibberella zeae* necessary for spore discharge. Heather Hallen[1], Brad Cavinder[1], and Frances Trail[1,2]. [1]Department of Plant Biology and [2]Department of Plant Pathology, Michigan State University, East Lansing, MI 48824-1312 hallenhe@msu.edu

We have identified two genes, encoding a calcium channel and a transcription factor, which are necessary for ascospore discharge in *Gibberella zeae*. Mutants of both genes are incapable of forcible spore discharge, but produce phenotypically normal perithecia and viable ascospores. Expression analysis of the mutants revealed that they do not regulate each other. Affymetrix GeneChip analysis yields hundreds of genes with 4-fold or greater expression difference between each mutant and wild-type ($p < 0.003$), suggesting that they play a significant role in both gene activation and repression in *G. zeae*. Interestingly, expression of many of the genes is affected in both mutants. These genes will allow us to begin to unravel ascus structure and function.

403. Defects in light regulation of sexual and asexual development in *Coprinopsis cinerea.* Wassana Chaisaena, Monica Navarro-Gonzalez, and Ursula Kües. Institute of Forest Botany, Georg-August-University, Göttingen, Germany

Fruiting body development in *Coprinopsis cinerea* is adapted to the light and dark phases of the normal day-night rhythm. If young developing structures (day 1 to day 4 of development) are transferred into constant darkness, normal cap and stipe differentiation arrests. Instead, the stipe base proliferates to give an elongated structure known as "etiolated stipe" or "dark stipe". Recessive mutations in genes *dst3* and *dst4* cause such phenotype in the normal day- night regime. These genes are distinctive from *dst1* and *dst2* identified before in Japan. From morphological analysis of the cap tissues of the etiolated stipes, *dst3* and *dst4* appear to act later in development (at day 3 and day 4 of development, respectively) than *dst1* and *dst2* (defects at day 2 of development). Unlike *dst3* and *dst4*, *dst1* and *dst2* also act in light-control of asexual sporulation. Only the *dst2* mutant is totally blind and forms etiolated stipes also under constant light incubation. Normally in constant light, fruiting body development is totally repressed. WC and MNG acknowledge scholarships from the Rajamangala University of Technology (Thailand) and CONACYT (Mexico), respectively. The Deutsche Bundesstiftung Umwelt financially supported the laboratory.

404. A constitutively activated Ras-protein has multiple effects on vegetative and sexual development in *Coprinopsis cinerea.* Prayook Srivilai, Monica Navarro-Gonzalez, and Ursula Kües. Institute of Forest Botany, Georg-August-University, Göttingen, Germany

A mutation in codon 19 of a cloned *ras* gene of *Coprinopsis cinerea* altering a glycine into a valine codon (ras^{val19}) gives rise to a constitutively activated GTPase. The ras^{val19} mutant allele has been transformed into different monokaryons of C. cinerea and found to affect the mycelium of monokaryons and, after mating, also of dikaryons. Growth rates were reduced, hyphae lost growth orientation, many abnormal short side-branches were formed and mycelial growth was invasive. ras^{val19} dikaryons had many abnormal unfused clamps at hyphal septa whose hyphal tips tended to further elongate, thereby passing the subapical pegs formed for clamp cell fusion. In fruiting body development, tissue formation within the primordia was altered. Gills appeared to be oversized compared to the upper stipe tissues and the plectenchyma in the pileus. Fully developed fruiting bodies were minute (1-1.5 cm) compared to normal 4-5 cm sized fruiting bodies. Basidiospore production was affected in quantity and quality. Many basidiospore were abnormal small. PS and MNG acknowledge scholarships from the Mahasarakham University (Thailand) and CONACYT (Mexico), respectively. The Deutsche Bundesstiftung Umwelt financially supported the laboratory.

405. Microarray-based gene expression profiling during fruitbody initiation in *Agaricus bisporus*. B. Herman, D.C. Eastwood, S. Sreenivasaprasad, A. Dobrovin-Pennington, R. Noble, K.S. Burton. Warwick HRI, University of Warwick, Wellesbourne, CV35 9EF, UK.

Homobasidiomycete *Agaricus bisporus* goes through a phase change from vegetative mycelium to reproductive growth to form fully differentiated mushroom fruitbodies. The molecular mechanisms controlling this process are poorly understood. In the early stages of the development, defined as mushroom initiation, a series of environmental parameters play key roles. These include a reduction in temperature and CO_2 concentration, heavy watering and biotic factors (e.g. microbial flora influencing the levels of eight-carbon volatiles). Little is known about how each parameter affects differentiation in *A. bisporus*and how the fungus senses and responds to the changes in the environment. This investigation is aimed at understanding the molecular basis of the phase change and to determine the effect of the environmental triggers on *A. bisporus* development and gene expression.Stage-specific cDNA suppression subtractive hybridisation procedure was used to identify genes (more than 300 unisequences) differentially expressed during the

mushroom initiation process. A database of more than 1000 *A. bisporus* genes representing the 300 unisequences and other cDNAs and ESTs was established. Bioinformatics was used to analyse these sequences and to design gene-specific probes which were processed through the Agilent Technologies eArray software to create a 60 base oligonucleotide microarray. The custom array is being used for comparative gene expression profiling during the initiation process in *A. bisporus* under different growth regimes.

406. Molecular biology of recognition between mating partners in *Mucor*-like fungi. Johannes Wostemeyer, Anke Burmester, Christine Schimek, Kornelia Schultze, Jana Wetzel. Institute of Microbiology, Chair of General Microbiology and Microbe Genetics, FSU-Jena, Germany.

Trisporoids are a general principle of communication between mating partners in mucoralean fungi. Their synthesis starts by cleavage of beta-carotene by a dioxygenase reaction and procedes via several reactions towards trisporic acid. Several of these reactions are specific for one of the mating types, resulting in the necessity for cooperative synthesis of the actice mating signal, trisporic acid. We have identified and cloned three of the genes encoding functions in the pathway from carotene to trisporic acid. Especially the *TSP1* gene for dihydromethyltrisporate dehydrogenase has been studied in detail with respect to its regulation. The gene product is active predominantly in the minus mating type in three different species analyzed, *Parasitella parasitica*, *Mucor mucedo* and *Absidia glauca*. Although the gene is controlled in a mating type-specific manner in all three organisms, the levels of its regulation differ considerably in these species between posttranscriptional reguation in the mycoparasite *P. parasitica*, posttranslational regulation in *M. mucedo* and a tight temporal regulation in *A. glauca*. In none of these species regulation of *TSP1* took place at the transcriptional level. Data on the regulation of the *TSP2* gene for the minus type- specific dihydrotrisporin dehydrogenase and the gene for the carotene dioxygenase will also be presented.

407. Characterisation of FlbB, a putative B-zip type transcription factor needed for early stages of conidiation induction in *Aspergillus nidulans.* Oier Etxebeste[1], Aitor Garzia[1], Min Ni[2], Reinhard Fischer[3], Jae-Hyuk Yu[2], Eduardo Espeso[4] and Unai Ugalde[1]. [1]Faculty of Chemistry, San Sebastian, Spain [2]FRI, Univ. Wisconsin, Madison [3]Dept Applied Microbiol.Univ. Karlsruhe, Germany [4]CIB, CSIC, Madrid, Spain

Conidiation in *Aspergillus nidulans* requires the activation of *brlA*, which in turn induces expression of other genes required for development. Genes expressed during vegetative growth participate in sensing the right conditions for *brlA* induction. These include genes collectively called *flb* (their mutants are fluffy with low brlA expression). Whilst one such mutant has been linked with G-protein signaling (*flbA*), others encode transcription factors with as yet unknown roles. We have cloned *flbB* as AN 7542.3 encoding a putative 427aa B-zip type transcription factor. The N-terminal B-zip DNA binding domain shows high similarity with that of Pap1, a transcription factor involved in adaptive responses to mild oxidative stress in *Schizosaccharomyces pombe*. In addition, FlbB also shares similarities with Pap1 at the C-terminal region, where two cysteine residues determine proper cellular localisation in response to mild oxidative stress. The pattern of expression, cellular distribution of FlbB, and relationship with other *flb* genes will be presented, with preliminary evidence that it may be responding to developmentally significant ROS signals.

408. The *bd* mutation in *Neurospora crassa* is a dominant allele of *ras-1* implicating ras-signaling in circadian output. Luis F. Larrondo, William J. Belden, Allan C. Froehlich, Mi Shi, Chen-Hui Chen, Jennifer J. Loros, and Jay C. Dunlap. Department of Genetics, Dartmouth Medical School, Hanover, NH 03755, USA. Luis.F.Larrondo@dartmouth.edu

The *band* (*bd*) mutation in *Neurospora crassa* has been an integral tool in the study of circadian rhythms for the last 40 years by enabling clear visualization of circadianly regulated conidia production (banding). The *bd* mutation was mapped using single nucleotide polymorphisms (SNPs), cloned, and determined to be a point mutation in the *ras-1* gene, changing threonine 79 to isoleucine. There is a defect in light-regulated gene expression in *ras-1*^{bd} mutants suggesting that the *Neurospora photoreceptor*, the WHITE COLLAR complex, is a downstream target in RAS signaling. There are also increases in the circadian regulated transcription of both *wc-1* and *fluffy* consistent with the idea that regulators of conidiation are elevated in *ras-1* ^{bd}. Comparison of *bd* with dominant active and dominant negative *ras-1* mutants and in biochemical assays of RAS function indicates that RAS-1 ^{bd} has only minor alterations in GTP/GDP exchange and GTPase activity. In addition to the *bd* mutation, we found that oxidative stress can also increase conidia production suggesting a link between generation of reactive oxygen species (ROS) and RAS-1 signaling. Altogether, the data indicate that multiple interconnected signaling pathways including RAS signaling and elevated ROS levels can elicit heighten conidiation and regulate the amplitude of circadian and light-regulated gene expression in Neurospora.

409. Evidence for a lectin-mediated defense of mushrooms against predators, parasites and pathogens. Martin Wälti[1], Anke Grünler[1], Michaela Bednar[1], Alex Butschi[2], Michael Hengartner[2], Markus Aebi[1] and Markus Künzler[1]. [1]Institute of Microbiology, ETH Zürich, Switzerland. [2]Institute of Zoology, University of Zürich, Switzerland.

The galectins CGL1 and CGL2 as well as the homologous lectin CGL3 of the homobasidiomycete *Coprinopsis cinerea* are strongly induced during sexual development and highly enriched in the fruiting body. Despite this differential expression pattern in time and space, neither ectopic expression during vegetative growth nor silencing of the respective genes had any obvious effect on fruiting. These results make a role of these lectins in mushroom development unlikely. In search of an alternative function, we found a pronounced toxicity of the galectin CGL2 towards the nematode *Caenorhabditis elegans*. The phenotype of the toxicity resembles the one of the nematotoxic crystal toxins from *Bacillus thuringiensis*. We are currently investigating the specificity and the mechanism of this CGL2-mediated toxicity. Moreover, preliminary data show that CGL3, which has a different sugar binding specificity than CGL2 and reveals no toxicity towards *C. elegans*, is able to specifically agglutinate some bacteria and to inhibit vegetative growth of some fungi. These results suggest that mushrooms and probably also other multicellular fungi contain, analogous to plants, a lectin-mediated defense against predators, parasites and pathogens that might be ecologically important. We are interested in the spectrum of defense molecules (with emphasis on lectins) and the regulation of this defense system in *C. cinerea* .

410. Role of WetA During Conidiation and Yeast Growth in the Thermo-dimorphic Fungal Pathogen *Penicillium marneffei.* David Cánovas[1], Mariam Ghosn, Luke Pase[2] and Alex Andrianopoulos. Department of Genetics, University of Melbourne, Victoria 3010, Australia [1] Current address: Departamento de Biotecnologia Microbiana, Centro Nacional de Biotecnologia - CSIC, Campus UAM - Cantoblanco, Madrid 28049, Spain. [2] Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3050, Australia.

***Penicillium marneffei* is a thermally dimorphic fungal pathogen of humans. At 25ºC the saprophitic form is filamentous in growth and composed of hyphal cells which are usually multinucleate and separated by incomplete septa. At 37ºC, the pathogenic form is an elongated unicellular and uninucleated yeast cell, which divides by fission. Besides the dimorphic switch, *P. marneffei* can also undergo asexual development (conidiation) at 25ºC resulting in the production of specialized structures called conidiophores, which bear the infectious form (conidiospores). The different cell types produced during the developmental programmes are strictly controlled. We cloned the homologue of the *Aspergillus nidulans* conidiation gene *wetA* from *P. marneffei*. Expression of *wetA* is dependent on the developmental transcriptional activators BrlA and AbaA during conidiation, but it is only AbaA- dependent during yeast growth. The *wetA* gene is not expressed during vegetative growth at 25ºC. Accordingly, a deletion of the gene showed morphological defects during conidiophore development and yeast growth but not during hyphal growth. A proportion of the conidiophores displayed abnormal morphologies, including cotton-ball shapes, aberrant cell types and multinucleated conidia. Over time conidial viability decreased and conidia displayed inappropriate germination. At 37ºC a proportion of yeast cells were multinucleated. The *wetA* deletion mutant yeast cells were generally shorter in length than wild type cells, had an increased tendency to lyse, and were more sensitive to SDS. The phenotype of the deletion strain at 37ºC can be explained by defects in cell wall/membrane structure and this is supported by TEM studies.**

411. Mechanisms by which the adhesin Flo11 determines morphology in *S. cerevisiae* . Yang Yang, Sungsu Lee and Anne M. Dranginis. Department of Biological Sciences, St. John's University, Queens, NY. drangina@stjohns.edu

Flo11, a cell wall adhesion molecule, is an important determinant of cellular morphogenesis and development in *S. cerevisiae*. In various strains of yeast, Flo11 may be required for pseudohyphae formation, biofilm formation, formation of mats containing specialized hub and spoke structures on semi-solid medium, and unusual colony morphologies. We find, however, that only a subset of these phenotypes are displayed by any given Flo11-expressing strain. In strain Sigma 1278b, for example, Flo11 is required for the formation of the hub and spoke structures but this strain does not flocculate. Strain *S. cerevisiae var. diastaticus*, on the other hand, requires FLO11 for flocculation but does not form hub and spoke structures. The Flo11-dependent phenotypes of strain SK1 include the development of a lacy colony morphology which is not exhibited by other strains. We are using these differences in strain-specific Flo11 phenotypes to investigate the mechanisms by which this adhesin determines morphology. In one approach, the domains of Flo11 have been separately purified and tested in vitro for adhesion to cells and other substrates. The amino terminal domain has proven to be the adhesion domain, and its cellular receptor is the mannosylated central domain of Flo11 on adjacent cells. Primary sequence differences in these domains correlate with phenotypic differences in some strains; gene- swap experiments are being used to determine whether these sequence differences suffice to confer phenotypic differences. Levels of expression and subcellular distribution of Flo11 in each strain are also being determined.

Animal and Plant Pathogens; Fungal-Host Interactions

412. Modulating the occurrence and distribution of ROS during nitrogen deprivation and early developmental stages of *Colletotrichum acutatum* infecting strawberry. Sigal Horowitz Brown[1,2], Oded Yarden[1], Natan Gollop[3,] Songbi Chen[3], Aida Zveibil[2], and Stanley Freeman[2]. [1]Dept. of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100; [2]Dept. of Plant Pathology, and [3]Dept. of Food Science, ARO, The Volcani Center, Bet Dagan 50250, Israel. Freeman@volcani.agri.gov.il

Significant alterations in the abundance of different proteins were found during wildtype *Colletotrichum acutatum* appressoria formation, under nitrogen limiting conditions or complete nutrient supply, when compared to a nonpathogenic mutant. CuZn superoxide dismutase and glutathione peroxidase were up-regulated at the appressoria formation stage and nitrogen limiting conditions, compared to growth in complete nutrient supply, whereas abundance of bifunctional catalase was up-regulated predominantly at the appressoria formation stage. Fungal ROS (reactive oxygen species) was detected within germinating conidia during strawberry host pre-penetration, penetration and colonization stages. Application of an exogenous antioxidant quenched ROS production and reduced appressoria formation frequency. A general up-regulation in metabolic activity during appressoria formation and under nutrient deficiency conditions was observed. Glyoxylate cycle and lipid metabolism–related proteins (malate dehydrogenase, formate dehydrogenase, and acetyl coA acetyltransferase) showed enhanced levels during the appressoria production phase in contrast to down-regulation of isocitrate dehydrogenase. This study indicates that developmental processes occurring under nutritional deprivation in *C. acutatum* infecting strawberry are facilitated by metabolic shifts which may be mediated by fungal ROS production.

413. Development of a Molecular Genetic Linkage Map for *Colletotrichum lindemuthianum* and Segregation Analysis of Two Avirulence Genes. Francisco Luna-Martínez[1], Raúl Rodríguez- Guerra[2], Mayra Victoria-Campos[1], June Simpson[1]. [1] Department of Genetic Engineering, CINVESTAV, Unidad Irapuato, A.P. 629, Irapuato, Guanajuato, México. [2] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), A.P. 112; Celaya, Guanajuato, México. jsimpson@ira.cinvestav.mx

A framework genetic map was developed for *Colletotrichum lindemuthianum*, the causal agent of anthracnose of common bean (*Phaseolus vulgaris L.*). This is the first genetic map for any species within the family Melanconiaceae and the genus *Colletotrichum* and provides the first estimate of genome length for *C. lindemuthianum*. The map was generated using 106 haploid F1 progeny derived from crossing two Mexican isolates differing in two avirulence genes (*AvrclMex* and *AvrclTO*). The map comprises 165 AFLP markers covering 1897 cM with an average spacing of 11.49 cM. The markers are distributed over 19 major linkage groups containing between 5 and 25 markers each and the genome length was estimated to be 3241 cM. *AvrclMex* and *AvrclTO* segregate in a 1:1 ratio supporting the gene for gene hypothesis for the incompatible reaction between *C. lindemuthianum* and *P. vulgaris*, but could not be incorporated into the genetic map. This map forms the basis for the development of a more detailed *C. lindemuthianum* linkage map which would include more markers and allow the location of genes previously characterized in this species.

414. The Unfolded Protein Response and the transcription factor Hac1p, in *Candida albicans*. Tithira T. Wimalasena[1], Thomas Guillemette[1, 3], Andrew Plumridge[1], Brice Enjalbert[2], Alistair J. P. Brown[2] and David B. Archer[1]. [1]School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, UK [2]Aberdeen Fungal Group, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK [3]Present address: UMR 77 Pathologie Vegetale, Faculte des Sciences, Université d'Angers, France

The Unfolded Protein Response (UPR) is a stress response that regulates the folding of proteins in the endoplasmic reticulum and the delivery of secretory proteins to the cell surface and exterior. *Candida albicans* is an opportunistic human fungal pathogen: its morphology and the secretion of enzymes and cell surface components are important factors in its virulence. We therefore aimed to describe the UPR in *C. albicans* and to determine its importance in nitrogen-dependent processes in *C. albicans*, such as the yeast-mycelial transition. In *S. cerevisiae*, the UPR induces the dimerization of a ER-resident type I transmembrane protein, Ire1p which activates splicing of *HAC1*^{u}, to *HAC1*^{i} mRNA by excising a 252-bp non- conventional intron towards the 3'end of the mRNA, which produces the functional Hac1p. This activates transcription of UPR-sensitive genes via direct binding to the cis-acting UPR element (UPRE) to activate its target genes. In *Aspergillus niger*, a similar mechanism is observed, but the intron is limited to 20-bp. In addition to this unconventional splicing in *A. niger* , a 5'-UTR truncation occurs which affects the size of the transcripts. Here we report the characterization of the gene encoding Hac1p in *C. albicans*. In this study, the UPR was induced by treating *C. albicans* yeast cells with dithiothreitol (DTT) and tunicamycin. The analysis of cDNA made from *HAC1* mRNA shows that there is a difference between the HAC1^{i} and HAC1^{u} towards the 3' end of the mRNA: HAC1^{i} mRNA lacks a 19-base intron that is present in *HAC1*^{u}. Furthermore, we deleted both copies of the *HAC1* gene in *C. albicans* to create a null mutant strain. We confirm the role of the UPR in the delivery of cell surface proteins and show also that the UPR has a role in determining the morphology of *C. albicans* .

415. *Aso1* is required for anastomosis and pathogenicity in *Alternaria brassicicola.* T.K. Mitchell[1], K. D. Craven[2], C. Yangrae[3], C. B. Lawrence[3]. [1]North Carolina State University, Dept. of Plant Pathology, Raleigh, NC 27695; [2]Samuel Roberts Noble Foundation, Ardmore, OK; [3]Virginia Bioinformatics Institute, Blacksburg, VA.

Vegetative fusion, or anastomosis, has been documented for many fungal species and provides an efficient conduit for the movement of nutrients, proteins, signaling molecules, and even viruses through a colony. Using nitrate-utilization mutants

of the necrotrophic plant pathogen *Alternaria brassicicola* and two related species, we characterized anastomosis capacity and investigated the possibility of an operating vegetative incompatibility system. All eight *A. brassicicola* isolates tested, as well as *Alternaria mimicula* and *Alternaria japonica*, were capable of self- anastomosis, with only two isolates capable of non-self fusion. In no case did we observe cell death or the development of a barrage between incompatible isolates. Interrogation of the genome sequence resulted in the identification of *het* and *so-1* homologs. The het genes are central to the vegetative compatibility system in *Podospora anserina*. Functional characterization of our deletion mutant of *aso-1* (*Alternaria so-1*) showed a loss of the ability to undergo self-anastomosis. Additionally, the deletion mutants were no longer pathogenic on cabbage leaves. Here we present our analysis of the *aso-1* locus, its functional characterization, and a model of how vegetative compatibility is functioning in this important plant pathogen.

416. Interaction with plants stimulates mating of the human fungal pathogen *Cryptococcus neoformans*. Chaoyang Xue[1], Yasuomi Tada[2], Xinnian Dong[2], and Joseph Heitman[1]. [1]Department of Molecular Genetics and Microbiology, [2]Department of Biological Science, Duke University, Durham, NC 27710. Email: xuecy@duke.edu

***Cryptococcus* species such as *C. neoformans* and *C. gattii* are globally distributed human fungal pathogens that primarily afflict immunocompromised individuals. How and why this human fungal pathogen associates with certain plant species, such as *Eucalyptus* trees, in its environmental niche has been mysterious. Here we established defined *Cryptococcus-Arabidopsis* and *Cryptococcus-Eucalyptus* interaction systems. Fungal cell proliferation and mating occurred on plant surfaces. The presence of plants (*Arabidopsis* or *Eucalyptus*) stimulated fungal mating even under constant light conditions, indicating that plant materials overcome the known inhibition of mating by light. Mating efficiency of *C. gattii* strains was markedly enhanced by the presence of plants. Completion of the sexual cycle in nature enables production of basidiospores - the suspected infectious propagule. On the plant side, dwarfing and chlorosis of *Arabidopsis* were often observed following infection with a fungal mixture of two opposite mating type strains under sterile growth conditions. The plant jasmonate- dependent defense pathway was induced by fungal infection, while the salicylate-dependent defense pathway was partially repressed. These findings reveal that *Cryptococcus* can parasitically interact with plants, compromising pathogen- induced resistance mechanisms to gain benefit from plants, especially during mating. Plant derived materials including myo-inositol and indole-3-acetic acid (IAA) have been discovered to stimulate mating, which may explain the association in nature between plants and this human fungal pathogen. The study of how fungal cells sense plant signals is underway.**

417. Differences in mating of *Cryptococcus* species on pigeon guano defines their ecological niches. Kirsten Nielsen, Anna Lisa De Obaldia, and Joseph Heitman. Department of Molecular Genetics and Microbiology Duke University Medical Center, Durham, NC

***Cryptococcus neoformans* is a pathogenic yeast that predominantly infects immunocompromised individuals. *C. neoformans* exists in two varieties - *grubii* (serotype A) and *neoformans* (serotype D), and is commonly isolated from pigeon guano (PG). By contrast, *Cryptococcus gattii* often infects immuncompetent individuals, and has been traditionally associated with other environments, including eucalyptus trees. *C. neoformans* and *C. gattii* diverged ~ 40 million years ago. The ecological niche a species occupies is determined by resource requirements and the physical conditions necessary for survival. The ecological niche to which an organism is most highly adapted is the realized niche, while the complete range of habitats the organism can occupy is considered the fundamental niche. To determine whether *C. neoformans* and *C. gattii* occupy different ecological niches, we examined the growth and development of these species on PG. This study shows that PG can support growth of *C. neoformans* and *C. gattii*. However, we found that *C. neoformans* exhibits prolific mating on PG, while mating of *C. gattii* is dramatically inhibited. The observation that *C. neoformans* has adapted to complete the lifecycle on pigeon guano suggests that PG represents a realized niche of *C. neoformans* but not *C. gattii*.**

418. The role of the small GTPase Rheb in the control of the development of the phytopathogenic fungus *Botrytis cinerea*. Heber Gamboa Meléndez[1], Weronika Podlesna[1], Michel Droux[2] and Géraldine Mey[1]. [1]Laboratoire de Pathogénie des Champignons Nécrotrophes, Université Claude Bernard- Lyon 1, UMR CNRS-INSA- UCBL 5122, 10 rue Dubois, Bât Lwoff, 69622 Villeurbanne Cedex, France. E-mail: hgamboa@univ-lyon1.fr [2] Laboratoire Mixte CNRS/Bayer CropSciece, Centre de Recherche de la Dargoire, Bayer CropScience, 14-20 rue Pierre Baizet, 69263, Lyon, France

The success of the infection process partly relies on the ability of fungal pathogens to adapt their metabolism to the use of the nutrients present in the host. Necrotrophic fungi have expended this skill to the complete colonization of the target plant subsequent to the enzymatic maceration of plant tissues. The nutrients released during the degradation process may both be included in the anabolic reactions required for the parasitic growth and act as signal molecules. The growth and the differentiation of eukaryotic cells is regulated via the TOR (Target of Rapamycin) dependant signalling cascade which seems to be conserved from yeast to humans: in the presence of extracellular nutrients, the small GTPase Rheb (Ras homologue enriched in brain) acts as a molecular switch and activates the kinase TOR. We investigated the role of the TOR pathway in the control of the growth of necrotrophic fungi, using as a model *Botrytis cinerea*, the causal agent of the

gray mold disease on more than 200 plant species. RNA interference was used to decrease the expression of the gene encoding *Rheb* in *B. cinerea*. The saprophytic growth of the rheb transformants appeared affected: the growth and the sporulation rates were reduced. The infection assays showed that the rheb mutants were less virulent than the wild type, indicating that the parasitic growth was also altered. Mutation of the gene *rhb1* in *Saccharomyces cerevisiae* (Rheb orthologue) increases the uptake of amino acids. Complementation experiments of the yeast *rhb1* mutant revealed that Rheb from *B. cinerea* restored the down regulation of certain but not all amino acid transport functions. The involvement of Rheb in the control of amino acid uptake in *B. cinerea* is currently further investigated using HPLC experiments and expression studies of transporter encoding genes by qRT-PCR. Taken together, these results will contribute to a better understanding of the role of Rheb in the control of amino acid metabolism and its involvement in the necrotrophic development of *B. cinerea.*

419. Mannitol is required for asexual sporulation in *Stagonospora nodorum*. Waters, Ormonde D.C., Solomon, P.S., Trengove, R.D. and Oliver, R.P. ACNFP, SABC, DHS, Murdoch University, Perth 6150, WA, Australia.

The physiological role of the mannitol cycle in the wheat pathogen *S. nodorum* has been investigated by reverse genetics and metabolite profiling. A putative mannitol 2-dehydrogenase gene was cloned and disrupted. The resulting strains lacked all detectable mannitol dehydrogenase activity. The *mdh1* strains were unaffected for mannitol production but surprisingly, were still able to utilize mannitol as a sole carbon source suggesting a hitherto unknown mechanism for mannitol catabolism. The mutant strains were not compromised in their ability to cause disease or sporulate. A previously developed mannitol 1-phosphate dehydrogenase disruption construct was introduced into the *mdh1* background, resulting in a strain lacking both enzyme activities. The *mpd1mdh1* strains were unable to grow on mannitol and produced only trace levels of mannitol. The double mutant strains were unable to sporulate *in vitro* when grown on minimal medium for extended periods. Deficiency in sporulation was correlated with the depletion of intracellular mannitol pools. Significantly sporulation could be restored with the addition of mannitol. Pathogenicity of the double mutant was not compromised, although like the previously characterised *mpd1 mutants*, the strains were unable to sporulate *in planta*. These findings question not only the currently hypothesised pathways of mannitol metabolism, but also identify for the first time that mannitol is required for sporulation of a filamentous fungus.

420 . The role of the fungal extracellular matrix in lesion development on plants. Giovanna Sassi, Natalie Catlett, Charlotte Bronson, and B. Gillian Turgeon. Cornell University, Department of Plant Pathology, gs242@cornell.edu

Polysaccharide capsules of fungi are virulence factors for several fungal-host pathosystems. The extracellular matrix (ECM) of the corn pathogen *Cochliobolus heterostrophus* surrounds germ tubes, appressoria, and plant-colonizing hyphae. A UV-generated *ecm1* mutant shows greatly reduced virulence on corn compared to wild type. Pre- penetration (attachment, spore germination, appressorium formation) and penetration activities are all normal suggesting the mutant is perturbed in plant colonization. The *ecm1* mutation has been mapped by AFLP/ RFLP analysis to chromosome 4 (Zhu *et al.* Genome 1998). Genome sequencing identified two large supercontigs flanking the *Ecm1* locus with the closest RFLP markers about 20 cM away. To provide closer markers for cloning by chromosome walking, the hygromycin B and nourseothricin resistance genes (*hygB and NAT*) were introduced at the proximal supercontig ends. As a second approach to cloning *ECM1*, the *Stagonospora nodorum* genome was queried with the proximal ends of the supercontigs flanking *ECM1*; a single supercontig was identified that spans the two *C. heterostrophus* contigs. This contig carries 3 candidate *ECM1* encoding genes. We hypothesize that ECMs play a role in plant pathogenesis by facilitating both virulence factor delivery and host response evasion.

421. Transcriptional adaptation of *Mycosphaerella graminicola* to programmed cell death (PCD) of its susceptible wheat host. John Keon[1], John Antoniw[1], Raffaella Carzaniga[2], Siân Deller[1], Jane L. Ward[3], John M. Baker[3], Michael H. Beale[3], Kim Hammond-Kosack[1] and Jason J. Rudd[1]. [1]Wheat Pathogenesis Programme, Plant-Pathogen Interactions Division, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK[2]Centre for Molecular Microbiology and Infection, Department of Infectious Diseases, South Kensington Campus Imperial College London, London. SW7 2AZ, UK[3]National Centre for Plant and Microbial Metabolomics, Rothamsted Research, West Common, Harpenden, Herts. AL5 2JQ E-mail address: Jason.rudd@bbsrc.ac.uk

Many important fungal pathogens of plants spend long periods (days to weeks) of their infection cycle in symptomless association with living host tissue followed by a sudden transition to necrotrophic feeding as host tissue death occurs. Little is known about either the host responses associated with this sudden transition or the specific adaptations made by the pathogen to invoke and/or tolerate it. Here we describe the host responses of wheat leaves infected with the Septoria Leaf Blotch pathogen, *Mycosphaerella graminicola* during the development of disease symptoms, and use microarray transcription profiling to identify adaptive responses of the fungus to its changing environment. We show that symptom development on a susceptible host genotype has features reminiscent of the hypersensitive response (HR), a rapid and strictly localized form of host programmed cell death (PCD) more commonly associated with disease resistance

mechanisms. The initiation and advancement of this host response is associated with a loss of cell membrane integrity and dramatic increases in apoplastic metabolites and fungal growth rate. Microarray analysis of the fungal genes differentially expressed before and after the onset of host PCD supports a transition to more rapid growth. Specific physiological adaptation of the fungus is also revealed with respect to membrane transport, chemical and oxidative stress mechanisms and metabolism. Our data support the hypothesis that host plant PCD plays an important role in susceptibility towards fungal pathogens with necrotrophic lifestyles.

422. Functional Characterisation of Cellulose Synthases from *Phytophthora infestans* using Proteomics and RNA interference. Laura J.Grenville-Briggs[1], Vicky L. Anderson[1], Catherine R. Bruce [1], Anna O. Avrova[2], Stephen C. Whisson[2], Paul R. J. Birch[2], and Pieter van West[1]. [1] Aberdeen Oomycete Group, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK. p.vanwest@abdn.ac.uk [2] Plant-Pathogen Interactions Programme, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK

The appressorial stage of the interaction between the late blight pathogen, *Phytophthora infestans*, and its hosts is one of the first points in which direct contact between the pathogen and the plant occurs via the formation of specialised infection structures, incl. the appressorium, penetration peg, and the infection vesicle. We have identified proteins that are abundant in appressoria using proteomics, one of which is a cellulose synthase (*Pi-Cel1*). Three other cellulose synthase genes, *Pi-Cel2*, *Pi-Cel3*, and *Pi-Cel4* were identified within the *P. infestans* EST collections. Real time RT-PCR reveals that all four genes are up-regulated in germinating cysts with appressoria. Gene silencing studies of the cellulose synthases perturb the morphology and structure of appressoria. Also culturing germinated cysts in the presence of cellulose synthase inhibitors resulted in identical changes in appressoria structure and morphology. Pathogenicity studies employing *P. infestans* zoospores in the presence of the cellulose synthase inhibitor resulted in greatly reduced infection rates.

423. Pathogenesis-related developmental processes in the rice blast fungus *Magnaporthe grisea.* Ane Sesma[1], Sara Tucker[1], Steve Lenhert[2]; Bruno Moerschbacher[2] and Anne Osbourn[3]. [1]Dept. of Disease and Stress Biology, John Innes Centre NR4 7UH Norwich, UK; [2]Westfälische- Wilhelms-Universität Münster, Hindenburgplatz 55, D-48143 Münster, Germany; [3]Dept. of Metabolic Biology, John Innes Centre NR4 7UH Norwich, UK; e-mails: sesma@bbsrc.ac.uk; Sara.Tucker@bbsrc.ac.uk; bruno.moerschbacher@uni-muenster.de; anne.osbourn@bbsrc.ac.uk

Host infection and virulence is commonly associated with dimorphism in fungal pathogens. *M. grisea* develops an invasive hyphal growth to colonise the epidermal cells which contain different morphological features from the fungal filaments produced on the leaf surface. Interestingly, we have found that the dimorphic transition which *M. grisea* undergoes in order to invade the plant cells can also be induced on smooth hydrophilic polystyrene (PS-PHIL) surfaces. This developmental switch is dependent on PMK1, a mitogen-activated protein kinase required by *M. grisea* to infect leaves and roots. To date 2,800 insertional transformants have been screened for abnormal growth on PS-PHIL surfaces. Two groups of mutants with different phenotypes have been identified. In the first group the mutants show hyper branched and accelerated growth compared to the wild-type. The other group of mutants lack the ability to undergo the developmental switch on PS-PHIL surfaces. Several mutants from both groups are defective in plant infection. This new system offers an easy and fast method to screen for mutants unable to infect plants.

424. Colonization of maize roots by *Colletotrichum graminicola* leads to symptomless systemic colonization of above ground plant tissues. Verónica M. García, Brian D. Shaw, Michael R. Thon and Serenella Sukno. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX. 77843 ssukno@tamu.edu

Anthracnose of maize is caused by the filamentous fungus, *Colletotrichum graminicola*, (Ces.) G. W. Wils. The disease can affect most plant tissues though the stalk rot and seedling blight forms of the disease are the most economically damaging. Little is known about its ability to infect roots. To better understand the process of root colonization, we constructed a GFP-expressing strain of, *C. graminicola*, and used it to perform a detailed histological study of plant tissue colonization upon root infection. Structures produced by other root pathogenic fungi were observed, including runner hyphae, hyphopodia and microsclerotia. A mosaic pattern of infection resulted from specific epidermal and cortical cells becoming infected by intercellular hyphae while surrounding cells are uninfected. Interestingly, falcate conidia, normally restricted to acervuli, were also found filling epidermal cells and root hairs. We observed the development of several fungal structures that may serve as survival structures that enable the fungus to over- winter. The microsclerotia produced in culture were able to infect plant roots, demonstrating that the structures were viable and could serve as a source of inoculum. Twenty-eight percent of plants challenged with soil-borne inoculum became infected in above ground plant parts (stem and/or leaves) indicating that root infection can lead to asymptomatic systemic colonization of the plants. These observations suggest that root infection may be an important component of the maize anthracnose disease cycle.

425. A mutant of *Leptosphaeria maculans* unable to produce the toxin sirodesmin PL has reduced pathogenicity on stems of *Brassica napus.* Candace E. Elliott, Donald M. Gardiner*, Anton Cozijnsen, Georgina Thomas, Angela Van de Wouw and Barbara J. Howlett. The School of Botany, University of Melbourne, VIC 3010 Australia. *Current address: CSIRO Plant Industry, St. Lucia, Qld, 4072 Australia.

Sirodesmin PL is a non host-selective phytotoxin produced by the ascomycete *Leptosphaeria maculans*, which causes blackleg disease of canola (*Brassica napus*). This toxin belongs to the epipolythiodioxypiperazine class of fungal secondary metabolites and its biosynthesis involves a cluster of 18 co-regulated genes. Disruption of the two module non-ribosomal peptide synthetase gene (*sirP*) in this cluster prevents the production of sirodesmin PL. Loss of sirodesmin in the *sirP* mutant did not affect growth or fertility *in vitro*, but resulted in an isolate with less antibacterial and antifungal properties than the wild type isolate. While this sirodesmin-deficient *sirP* mutant was able to infect cotyledons of *B. napus* as efficiently as wild type, it was less able than wild type to infect stems. The expression of two genes in the cluster, *sirP* and the ABC transporter (*sirA*) was studied *in planta* through the use of isolates containing fusions of the green fluorescent protein (GFP) with their promoters. GFP fluorescence of both isolates was observed during infection of cotyledons and of stems, and was especially strong in pycnidia and in hyphae growing in necrotic stem tissue. This pattern of gene expression was consistent with the distribution of sirodesmin *in planta*. Reverse phase high performance liquid chromatography and ElectroSpray Ionization – Mass Spectrometry/ Mass Spectrometry showed the presence of sirodesmin PL in infected cotyledons and stems of *B. napus*. This is the first report of the detection of sirodesmin PL *in planta*.

426. Identification of differentially expressed proteins of *Verticillium longisporum* during infection of *Brassica napus* (Rapeseed). Seema Singh[1], Susanna A. Braus-Stromeyer[1], Oliver Valerius[1], Sven Krappmann[1], Gerhard H. Braus[1]. [1]Institute of Microbiology and Genetics, Department of Molecular Microbiology & Genetics, George-August University, Göttingen, Germany. Tel: +49-551-393817, e-mail: ssingh1@gwdg.de

***Verticillium longisporum* is a devastating soil-borne pathogen on rapeseed crops causing vascular wilt disease. During growth of *V. longisporum* in the xylem vessels, it is likely that some proteins of *V. longisporum*, important for fungal growth or pathogenesis, may be differentially expressed on receiving possible defense signals from the host-plant. This project aims at the identification of such differentially expressed proteins. It involved quantitative comparison of proteomes of cell extracts of different culture conditions i.e. the fungus cultured with or without xylem sap from *Brassica napus* by 2D-PAGE. ESI-MS/MS identification of the digested 2-D spots was employed later. Some differentially expressed protein spots were successfully identified. Seven protein spots are up-regulated and five are down- regulated. These identified proteins will serve as a basis for the cloning of corresponding coding genes. Reporter constructs of identified genes, tagged with GFP and integrated in *V. longisporum* will be tested on induction by xylem sap and *in planta*. This will establish a suitable system for the identification of the possible fungus-plant interaction by changed gene expression.**

427. Iron source preference and regulation of iron uptake for *Cryptococcus neoformans.* Won Hee Jung, Tianshun Lian, Anita Sham and James W. Kronstad. Michael Smith Laboratories, University of British Columbia, 2185 East Mall, Vancouver, B.C., V6T 1Z4, Canada whjung@interchange.ubc.ca

Iron is required by virtually all organisms for their growth and proliferation. The competition for iron between host and pathogen is a critical aspect of many infectious diseases. The availability of iron in mammalian tissues and fluids is maintained at extremely low levels (10^{-18} M) by iron-binding proteins such as transferrin (Tf). However, pathogenic microbes require 10^{-6} to 10^{-7} M to accomplish key metabolic processes. Thus, they must steal iron from extracellular proteins by binding ferrated Tf, by elaborating siderophores, or by degrading hemoglobin or other iron containing proteins. We have been studying iron uptake and regulation in the human pathogen *C. neoformans*. These studies included global analyses of iron-regulated gene expression using serial analysis of gene expression and microarrays. Functional studies of target genes led to the characterization of Sit1, a siderophore transporter of the non-reductive iron uptake system, and Cir1, a global iron regulator that controls the regulon of genes for iron acquisition. More recently, we have characterized components of the reductive iron uptake system to achieve a more thorough understanding of iron utilization in *C. neoformans*. Here we present identification and functional characterization of *CFT1* (*Cryptococcus* Fe transporter). Reverse genetic analysis revealed that the gene is responsible for ferric iron uptake and transferrin utilization *in vitro*. Importantly, a mutant lacking *CFT1* displayed significant attenuation of virulence suggesting that *C. neoformans* utilizes transferrin as the primary iron source in host cells. Regulatory mechanisms for *CFT1* and other iron-responsive genes have also been studied in various strains including mutants lacking *CIR1* or components of the cAMP pathway. Our results indicate that *CFT1* is a direct downstream target of Cir1 and that the cAMP pathway also influences iron utilization in *C. neoformans*.

428. Mapping of the avirulence gene, *AvrLepR3* in the blackleg fungus, *Leptosphaeria maculans*. Angela Van de Wouw[1] and Barbara Howlett[1]. [1]School of Botany, The University of Melbourne, Parkville, VIC, 3010 Australia

The blackleg fungus, *Leptosphaeria maculans*, is the most serious pathogen of canola (*Brassica napus*) world wide. Current practices to control this disease include breeding disease resistance genes into canola varieties and management such as rotations with other crops. Several disease resistance genes have been mapped in *B. napus* and the corresponding avirulence genes in *L. maculans* identified, and in two cases cloned. Disease resistance from a related *Brassica, B. sylvestris* conferred by a major gene, *LepR3*, has been bred into Australian canola varieties. However, within three years of commercial release, the frequency of virulent isolates in fungal populations has changed such that resistance has been overcome with up to 95% yield losses in some regions. We are mapping the avirulence gene, *AvrLepR3* in the pathogen corresponding to the *LepR3* resistance gene. Crosses have been set up between *L. maculans* isolates with either avirulent or virulent phenotypes on varieties with *LepR3*. More than 200 progeny have been generated and they segregate in a 1:1 ratio of avirulence:virulence. Using the raw sequence files from the *L. maculans* genome project (Genoscope, France), we are developing microsatellite markers and minisatellite markers. These are currently being tested on progeny to identify linked markers to *AvrLepR3*.

429. Molecular studies of the *Saprolegnia*-fish interaction. Andrew J. Phillips, Emma J. Robertson, Vicky L. Anderson, Chris J. Secombes and Pieter van West. Aberdeen Oomycete Group, University of Aberdeen, IMS, Foresterhill, Aberdeen, AB25 2ZD, UK. E-mail: p.vanwest@abdn.ac.uk

Oomycetes of the genus *Saprolegnia* are responsible for devastating infections of fish. The disease (Saprolegniosis) is characterized by visible white or grey patches of filamentous mycelium on the body or fins of freshwater fish and is of particular problem to aqua-cultural businesses. We are investigating the molecular mechanisms which enable *Saprolegnia* to successfully infect fish, the molecular processes that suppress host defenses during infection, and the nature of the pathogen/host interaction. To enable us to study the fish pathogen interaction we have developed an *in vitro* infection model. In this model system a cultured-monolayer of a primary fish cell-line (RTG-2) is infected with cysts of *S. parasitica*. This model has enabled us to harvested material from several stages of the interaction between fish and *Saprolegnia*, allowing us to investigate the kinetics of the infection using a range of molecular, microscopic and biochemical techniques. We are particularly interested in the early time-points of the interaction, and are studying the mechanisms which allow *Saprolegnia* to establish an infection, and the defensive mechanisms employed by the host. We are currently addressing the latter by conducting microarray studies to detect changes in the host transcriptome in response to infection by *S. parasitica*. Our latest findings will be presented.

430. Functional characterisation of putative SNARE proteins from the fish pathogen *Saprolegnia parasitica.* Emma J. Robertson[1], Vicky L. Anderson[1], Andrew J. Phillips[1], Chris J. Secombes[2], and Pieter van West[1]. [1]The Aberdeen Oomycete Group, College of Life Sciences and Medicine, University of Aberdeen, Foresterhill, Aberdeen, Scotland, UK. E-mail: p.vanwest@abdn.ac.uk. [2] Scottish Fish Immunology Research Centre, Aberdeen, Scotland,UK.

The oomycete *S. parasitica* causes Saprolegniosis, a serious fish disease whereby mycelium grows on incubating eggs, or into the fins and body of freshwater fish. Severe infection result in death of the host, due to an imbalance in osmoregulation. Very little is known about the molecular biology of *S. parasitica*, and pathogenicity is largely undetermined. To gain more information about which genes are expressed at the onset of an infection and during pathogen development, we constructed an cDNA library from the pre-infectious stage of the *S. parasitica* life cycle, namely cysts. This resulted in the isolation of two EST sequences with homology to syntaxin and synaptobrevin, both members of the SNARE protein superfamily. SNAREs are involved in vesicular transport and membrane trafficking and fusion in a range of organisms, and play a critical role at different stages of the organism's development. Consequently, it is anticipated that SNARE proteins may be involved in encystment of *S. parasitica* zoospores, an important stage for the organism's pathogenicity. Herewe present experiments to identify and characterise syntaxin and synaptobrevin from *S. parasitica*.

431. Identification of *Fusarium oxysporum* f. sp. *lycopersici* pathogenicity genes through *Agrobacterium*-mediated insertional mutagenesis. Caroline B. Michielse, Ringo van Wijk, Ben J.C. Cornelissen and Martijn Rep. Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands.

***Fusarium oxysporum* f. sp. *lycopersici* is a soil-born fungus that causes vascular wilt disease in tomato by penetrating the plant roots and colonizing the plant xylem vessels. In order to identify genes involved in pathogenicity 10,209 random insertion mutants were generated using T-DNA of *Agrobacterium tumefaciens* as an insertional mutagen. Currently, over 6800 mutants have been assessed in bioassays. So far, 16 non-pathogenic mutants and 65 mutants with reduced pathogenicity have been identified. Using Southern analysis, an estimate of the average T-DNA copy number was obtained for the entire collection, as well as the T-DNA integration pattern for all identified pathogenicity mutants. In**

addition, TAIL-PCR was used to isolate the genomic regions flanking the T-DNA of the pathogenicity mutants. Several known pathogenicity genes were identified, such as class V chitin synthase and phosphomannose isomerase. Work is in progress to identify, verify and characterize the remaining pathogenicity genes.

432. The impact of Rho-GTPases on polar growth, differentiation and pathogenesis in the grass pathogen *Claviceps purpurea*. Yvonne Rolke and Paul Tudzynski. Institut für Botanik, Westf.Wilh.Universitaet,Schlossgarten 3, Muenster, Germany

The infection of rye by *Claviceps purpurea* is an interesting model for directed growth of fungal hyphae in host tissue. *C. purpurea* infects the stigma and grows in a strictly directed, unbranched modus through the different tissues of the floret (following the pollen tube path), to tap the vascular tissue. We are interested in the signalling mechanisms which guide the pathogen during this early colonisation phase. Since the Rho-GTPases Cdc42 and Rac are known as key players in differentiation and in establishment of polarity in fungi we analysed their impact on the *C. purpurea*/rye system. Cpcdc42 is involved in this directed growth phase and controls branching and conidiation (Scheffer et al 2005). Deletion of the gene encoding the *C. purpurea* homologue of Rac resulted in a much stronger phenotype: Δcprac mutants grow in a three dimensional, corral like shape with shortened, hyperbranched hyphae; they are non-sporulating and apathogenic.Thus Cpcdc42 and Cprac have strong and partially opposite effects on differentiation and pathogenicity in *C.purpurea.* Deletion of the PAK kinase gene cpcla4 yielded a Δrac-like phenotype; since Y2H analyses confirned interaction of Cpcla4 with Cprac, these data indicate that CpCla4 is activated by Cprac. The influence of Cpcdc42, Cprac and Cpcla4 on gene expression and on the phosphorylation status of different MAP-kinases is currently analyzed. Scheffer et al.(2005) EC 4:1228-1238

433. *Agrobacterium*-mediated Transformation of *gfp* into *Muscodor albus*, an Endophytic, Gas-emitting Fungus. David Ezra and Tami Kroiter. Department of Plant Pathology and Weed Research, ARO, the Volcani center, Bet – Dagan 50250; Israel

***M. albus* is an endophytic fungus originally isolated from *Cinnamomum zeylanicum* from Honduras. This fungus produces a mixture of volatile organic compounds (VOCs) which is lethal to a wide variety of fungal and bacterial plant pathogens. *M. albus* has been the subject of a number of studies which have suggested the use of this fungus as a biological control agent against plant pathogens. Our study examines the possible use of *M. albus* for the control of systemic diseases of fruit trees. *M. albus* is completely sterile; no spores or fungal fruiting bodies could be observed under different laboratory conditions. Unlike plant pathogens that induce disease or plant defense responses, when inoculated into the host plant, symbiotic endophytes do not usually cause visible reactions. This may be a problem for the researcher trying to decide whether the plant was successfully infected by the endophyte or not. In order to properly study the establishment, development and progress of the endophyte in the host plant and host-endophyte interactions, methods for the identification and localization of endophytic microorganisms are needed. One approach is to attempt to re-isolate the endophyte from the plant. Another strategy involves generating specific PCR primers for the identification of the endophyte in the plant. A third approach involves the transformation of a reporter gene into the endophyte and the subsequent localization of its expression in the host plant. We have used all three of these methods in our study of *M. albus in planta*. This report will describe the *Agrobacterium*-mediated transformation of the reporter gene *gfp* into *Muscodor albus* hyphae.**

434. Molecular and cellular responses of *Alternaria brassicicola* to plant defense metabolites. Adnane Sellam[1,2], Anita Dongo[2], Thomas Guillemette[2], Pascal Poupard[2], Piétrick Hudhomme[3], and Philippe Simoneau[2]. [1] Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount, Montreal, Quebec, Canada [2] UMR PaVé N°77, Faculté des Sciences, 2Bd Lavoisier, F-49045 Angers, France [3] Chimie, Ingénierie Moléculaire et Matériaux, UMR CNRS 6200 - Université d'Angers, 2 Bd Lavoisier, 49045 Angers, France

***Alternaria brassicicola* is the causative agent of black spot disease of Brassicaceae belonging to *Brassica* and *Raphanus* genus. During host infection, *A. brassicicola* is exposed to high levels of antimicrobial defense compounds such as indolic phytoalexins and glucosinolates breakdown products. To investigate the transcriptomic response of *A. brassicicola* when challenged with brassicaceous defense metabolites, suppression subtractive hybridization (SSH) and Differential Display were performed. Following exposure to ITC, *A. brassicicola* displayed a response similar to that experienced during oxidative stress. Indeed, a substantial subset of differentially expressed genes was related to the glutathione (GSH) cycle and to other mechanisms involved in cell protection against oxidative damages. Generation of Reactive Oxygen Species (ROS) following the exposure of *A. brassicicola* to ITCs was clearly demonstrated using two different fluorogenic probes. Treatment of germinated condia with the phytoalexin camalexin induced the expression of another functional set of genes. Indeed, this phytoalexin appeared to activate a compensatory mechanism to preserve cell membrane integrity. Thus , among the camalexin-elicited genes, several were involved in sterol and sphingolipid biosynthesis. An uptake assay using the fluorigenic dye SYTOX green confirmed the alteration of *A. brassicicola* membranes during exposure to camalexin.**

During interaction, the upregulation of all fungal selected genes was observed during the infection of *Raphanus sativus* while only a subset was overexpressed during the incompatible interaction with *Arabidopsis thaliana* ecotype Columbia. The Glutathione S-transferase (GST) gene *AbGst1*, differentially expressed in response to ITCs, was functionally characterized. The obtained results point to a function of AbGst1p in ITC conjugation and neutralization, enabling *A. brassicicola* to tolerate ITC during host infection.

435. Towards a comprehensive metabolome of *Stagonospora nodorum.* Peter S. Solomon[1], R.D. Trengove[2] and R.P. Oliver[1]. [1]Australian Centre for Necrotrophic Fungal Pathogens and [2]Separation Science Laboratory, SABC, DHS, Murdoch University, WA 6150, Australia. Email: psolomon@murdoch.edu.au

***Stagonospora nodorum* is a potent pathogen of wheat causing yield losses throughout many parts of Australia, the United States and Israel. In an effort to develop strategies to control yield losses, the Australian Centre for Necrotrophic Fungal Pathogens (ACNFP) has taken a multi-discipline approach to understanding the molecular basis of the disease. Metabolomics is rapidly emerging as a fast and powerful complementary technique to genomics and proteomics, particularly for the dissection of previously generated targeted gene knockout strains. Several platforms currently exist to study metabolomics including GC-MS, GC-TOF-MS, NMR, CE-MS and FT-MS. Advantages and disadvantages are evident for each of these techniques. Recently, the Separation Science Laboratory at Murdoch University has acquired a suite of new instruments that will complement the existing metabolomics platforms available. Of particularly interest is a GC × GC-TOF-MS that allows two-dimensional separation prior to time-of-flight analysis. This new instrument provides greater sensitivity and vastly improved separation compared to existing GC-MS platforms. This poster will provide a comparison of the detectable metabolites extracted from *S. nodorum* using GC-MS and GC × GC-TOF-MS platforms.**

436. Functional analysis of the role of a stress-activated MAP kinase in the association between a fungal endophyte and its grass host. Carla Eaton[1], Simon Foster[2], Jeremy Hyams[1], and Barry Scott[1]. [1] Massey University, New Zealand. [2]Sainsbury Laboratory, Norwich, UK.

The association between the fungal endophyte *Epichloe festucae* and its grass host *Lolium perenne* is regulated by the multicomponent NADPH oxidase complex. This complex catalyses production of reactive oxygen species (ROS) from molecular oxygen. Previous work has shown that disruption of a number of components of this complex results in an abnormal plant interaction phenotype, in which hyphal growth is unregulated and the host undergoes premature senescence. In *Aspergillus nidulans*, disruption of the stress-activated MAP kinase SakA has been shown to affect regulation of the NADPH oxidase, suggesting a link between SakA and ROS signalling (Lara-Ortiz *et al*, 2003). This suggests that SakA may also have a role in controlling the association between *Epichloe festucae* and its grass host. The *sakA* gene has been cloned based on conserved microsynteny at *sakA* locus in a number of filamentous fungi. This gene was then disrupted and the mutant shown to display increased osmosensitivity in culture. The mutant also displays resistance to the phenyl-pyyrole fungicide fludioxonil, as has been seen in other filamentous fungi defective in the stress-activated MAP kinase. The *E. festucae sakA* mutant also displays an abnormal host interaction phenotype, suggesting a possible role for SakA in regulating the symbiosis.

437. *Magnaporthe grisea* Mstu1, an APSES protein homolog of *Aspergillus nidulans* StuA, is required for regulation of appressorial turgor generation. Marie Nishimura, Junji Fukada and Nagao Hayashi. National Institute of Agrobiological Sciences, Tsukuba, Japan.

APSES proteins are transcription factors with highly conserved basic helix-loop-helix (bHLH) DNA binding domains to regulate fungal differentiation during asexual and sexual developments. We have identified a deletion mutant of the APSES protein Mstu1 (Magnaporthe STUA homolog 1) in *M. grisea* that showed reduced conidiation and mycelial growth, and was defective in plant penetration even though it formed comparable number of melanized appressoria relative to the wild type. *M. grisea* penetrates plant cuticles using appressorial turgor, which is generated by accumulation of glycerol synthesized from conidial reserves such as glycogen and lipids. In *mstu1*, delay in transfer of conidial glycogen and lipids to appressoria, and in sequential catabolism of those conidial reserves was observed. Furthermore, appressorial turgor was insufficient for plant penetration in *mstu1*. A single point mutation introduced at the potential PKA phosphorylation site in Mstu1 (T112A) resulted in low appressorial turgor and a delay in mobilization of lipids, and in catabolism of glycogen and lipids in the engineered mutant. Our results indicate that Mstu1 is a downstream target of the PKA and controls mobilization of lipids, and catabolism of the glycogen and lipids during appressorial turgor generation, and that glycogen mobilization is regulated by Mstu1 in PKA independent manner.

438. A distinct class of RNA binding proteins in *Magnaporthe grisea* implicated in pathogenicity. Sara L. Tucker[1], Anne E. Osbourn[2] and Ane Sesma[1]. [1]Department of Disease and Stress Biology, [2]Department of Metabolic Biology, John Innes Centre, Colney Lane, Norwich, NR4 7UH, UK. (sara.tucker@bbsrc.ac.uk)

A random mutagenesis approach was used to generate a library of insertional mutants in M. grisea strain Guy-11. The mutant bank was screened using a root infection assay, which led to the identification of several candidate strains with a reduced pathogenicity phenotype. One mutant strain, RBP35, is considerably affected in its ability to cause disease on roots while there is a small reduction in virulence on the aerial parts of rice plants. The mutant shows a slight reduction in growth rate and is impaired in conidiation compared to the wild type strain. RBP35 has undergone insertional inactivation of a gene encoding a predicted RNA binding protein. Such proteins are characterised based on the presence of one or more RNA recognition motifs (RRMs). The RRM is a nucleic acid binding domain that can act together and/or in association with other domains which then contribute to protein cooperativity and/or affinity for ssRNA. Characterisation of RRM-containing proteins in other systems has revealed that they can have a wide subcellular distribution and various functions in pre-mRNA processing and splicing, RNA stability, sequestration and degradation, mRNA transport, rRNA metabolism, telomere metabolism and protein recognition. Our initial efforts have focused on defining this class of proteins in M. grisea. We have set out to determine the subcellular location of RBP35 and to identify other proteins with which it may interact.

439. Functional analysis of Nep1-like proteins in *Sclerotinia sclerotiorum*. Alicia Greenhill[1], Rebecca Barnett[1], Megan Drew[1], & Kim Plummer[1]. [1]La Trobe University, Melbourne, Australia. k.plummer@latrobe.edu.au

***Sclerotinia sclerotiorum* is an aggressive necrotrophic phytopathogen causing white mould in >400 plant species worldwide. *S. sclerotiorum* is estimated to cost the US agricultural industry in excess of $200 million pa. Effective control methods of *S. sclerotiorum* are lacking and no resistant crop cultivars have been identified. We are using the *S. sclerotiorum* genome sequence (Broad Institute) to investigate necrosis inducing proteins secreted during infection. Two candidates, SS1G_03080.1 (*SsNLP1*) and SS1G_11912.1 (*SsNLP2*), have high similarity to the *Fusarium oxysporum* necrosis and ethylene-inducing peptide 1 (Nep1). Nep1-like proteins (NLPs), ranging from 24-26 kDa, are conserved across a wide range of taxa including bacteria, fungi and oomycetes. Bioinformatic analysis indicated *SsNLP1* and *SsNLP2* encode putative proteins 246aa and 245aa in length, with predicted secretory signal peptides of 20aa and 19aa, respectively. SsNLP1 and SsNLP2 had significant amino acid sequence identity to NLP family members, and contained conserved cysteine residues. Semi- quantitative RT-PCR analysis indicated that *SsNLP1* and *SsNLP2* are differentially regulated *in vitro* and during infection of *Brassica napus*. Gene silencing and knockout studies are presently underway to determine the role of SsNLP1 and SsNLP2 in pathogenicity of *S. sclerotiorum*.**

440. ROS signalling in the grey mould *Botrytis cinerea*. Nora Temme, Nadja Segmueller and Paul Tudzynski Institut fuer Botanik, Westf.Wilhelms Universitaet Muenster, Germany

Reactive oxygen species (ROS) play a major role in plant-pathogen interactions. The necrotrophic plant pathogen *Botrytis cinerea* causes an oxidative burst on its host plants. Its virulence correlates with (and might even depend on) the intensity of this induced plant defence reaction. To analyse ROS signalling in *B. cinerea*, components of the oxidative stress response system were characterised. The stress-activated MAPK (SAPK) BcSak1, a homologue of Hog1, is phosphorylated under oxidative stress;.its deletion showed that in *B.cinerea* this MAPK cascade is essential for vegetative differentiation (conidiation) and pathogenicity (penetration). The Botrytis activator protein 1 (Bap1) is a bZIP transcription factor structurally related to Yap1 from yeast. Deletion of bap1 resulted in increased sensitivity to oxidative stress. Bap1 controls expression of typical oxidative stress response genes,e.g. bccat2 (catalase 2), bctrr (thioredoxine reductase) and bcglr (glutathione reductase). Only bccat2 is also under control of Bcsak1, indicating that Bap1 acts mostly independently of the SAPK signalling cascade. In contrast to bcsak1, bap1 is not essential for conidiation or virulence. BcHk1 is a histidine kinase homologous to Mak2/3 which are involved in the oxidative stress response in *Sch. pombe.* Deletion of bchk1 results in increased sensitivity to oxidative stress and to the dicarboximide iprodione; activation of BcSak1 by oxidative stress caused by low levels of H2O2 is affected by BcHk1. Analysis of the response regulator Bcrr1 (a homologue of Skn7/Prr1) is under way.

441. Characterization of extracellular phospholipase D activity in *Phytophthora.* Harold J.G. Meijer and Francine Govers. Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, Binnenhaven 5, NL-6709 PD, Wageningen, The Netherlands

Phospholipase D (PLD) catalyzes the hydrolysis of structural phospholipids, such as phosphatidylcholine, leading to the production of phosphatidic acid and a free headgroup. In comparison to other eukaryotes, *Phytophthora* spp. have a more complex and diverse set of PLD genes. Mammals have only one type of PLD called PXPH-PLD. Plants also have PXPH-PLDs but in addition they have a large family of C2-PLDs. In these proteins, PX, PH and C2 are the lipid- binding domains that precede the catalytic PLD domain. *Phytophthora* has one PXPH-PLD, lacks C2-PLDs, but has three additional novel PLD sub-families (Meijer and Govers 2006), one of which is a large family of 12 members that all have a signal peptide and are probably secreted. The substrates for these secreted PLDs could be phospholipids that reside in plant membranes, one of the barriers faced by the pathogen when it enters the host. To test the hypothesis that the

secreted PLDs play a role in the infection process, we first analyzed PLD activity in the pool of extracellular proteins secreted by *Phytophthora*. Here we present the first biochemical characterization of PLD activity present in extracellular fluid and of the substrate specificity. Meijer, H.J.G., and Govers, F. 2006. Genomewide analysis of phospholipid signalling genes in *Phytophthora* spp.: novelties and a missing link. Mol. Plant Microb. Inter. 19:1337-1347.

442. Comparative transcriptional profiling of *Blumeria graminis* development on host and non-host surfaces. Petro D Spanu, Maike Paramor, Calin Andras. Imperial College London

Blumeria graminis* f sp *hordei* is a fungus that causes powdery mildew on barley. When conidia land on barley epidermis, they germinate and grow following a very tightly controlled series of developmental stages: production of a short primary germ tube then a secondary germ tube that enlarges to form an appressorium, from which a penetration peg breaches the cell wall and grows into a feeding haustorium. Proliferation of the extracellular mycelium, and the associated secondary haustoria, eventually lead to massive production of conidia for further dispersal. On non- host plants, such as wheat, development is arrested early, at penetration or shortly thereafter. On artificial surfaces, e.g. cellophane or glass, development is aborted prior to differentiation of appressoria or secondary germ tubes respectively. In the work reported at this meeting, we have carried out a comparative analysis of the transcriptional profiles of over 2000 genes during early development on barley, wheat, cellophane and glass. We find genes whose regulation is specifically affected by the surfaces on which *Blumeria* grows. Surprisingly, *Blumeria* differentiates between barley and wheat surfaces well before differences are visble. We will report here on the genes that appear to characterise growth on host, non host and artificial surfaces. Our ultimate aim is to identify the common regulatory elements of these genes that are likely to be the target of networks controlling development of early stages of infection in *Blumeria.

443. Expression of a dominant active MgRas2 bypassed surface recognition signals for appressorium formation in *Magnaporthe grisea.* Xinhua Zhao, Chaoyang Xue, Yangseon Kim, and Jin-Rong Xu Department of Botany and Plant Pathology, Purdue University

Ras proteins are low molecular weight GTP-binding proteins that function as a molecular switch in signal transduction. We have identified two Ras homologues in *Magnaporthe grisea*. The *mgras1* deletion mutant had no detectable phenotypes but the efforts to generate a *mgras2* mutant failed, indicating that *MgRAS2* may be an essential gene. Expression of a dominant active MgRas2^{G23V} allele in the wild-type strain had no effect on vegetative growth but enhanced conidiation. Transformants expressing MgRas2^{G23V}(DA66) efficiently formed appressoria on both hydrophobic and hydrophilic surfaces. Interestingly, DA66 formed melanized appressorium- like structures on the tip of aerial hyphae. We then transformed the MgRas2^{G23V} construct into other appressorium-forming fungi. In the resulting transformants, appressorium-like structures were observed at hyphal tips. When an appressorium-specific reporter was transformed into DA66, GFP signals were observed in these appressorium- like structures. DA66 had an elevated intracellular cAMP level and enhanced phosphorylation of the Pmk1 MAP kinase. However, expressing MgRas2^{G23V} had no effect in the mutants that blocked the Pmk1 pathway. These data indicated that MgRas2 may function upstream from both the cAMP/PKA and Pmk1 MAP kinase pathways. The G23V mutation in MgRas2 enables *M.grisea* to bypass surface recognition and attachment signals for appressorium formation.

444. The application of laser microdissection to in planta gene expression profiling of the maize anthracnose stalk rot fungus *Colletotrichum graminicola.* Weihua Tang, Sean Couphlan, Edmund Crane, Mary Beatty, Dong Zhang and Jon Duvick . Shanghai Inst. Plant Phys. Eco.

Laser microdissection (LM) offers a potential means for deep sampling of a fungal plant-pathogen transcriptome during the infection process using whole-genome microarrays. Using a fluorescent protein-expressing fungus can facilitate the identification of fungal structures for LM. However, fixation methods that preserve both tissue histology and protein fluorescence, and that also yield RNA of suitable quality for microarray applications, have not been reported. We developed a microwave-accelerated acetone fixation, paraffin-embedding method that fulfills these requirements. We successfully used LM to isolate individual maize cells associated with *Colletotrichum graminicola* (AmCyan) hyphae at an early stage of infection. The LM-derived RNA, after two-round linear amplification, was of sufficient quality and quantity for global expression profiling using a fungal microarray. Comparing replicated LM samples representing an early stage of stalk cell infection with samples from in vitro-germinated conidia, we identified 437 and 370 *C. graminicola* genes showing significant up- or downregulation, respectively. We confirmed the differential expression of several representative transcripts by quantitative RT-PCR and documented extensive overlap of this dataset with a PCR-subtraction library enriched for *C. graminicola* transcripts in planta. Our results demonstrate that LM is feasible for in planta pathogen expression profiling and can reveal clues about fungal genes involved in pathogenesis.

445. Yeast LHS1 homologue, MoLHS1, plays multiple roles in the vegetative growth, asexual sporulation and pathogenicity of the rice blast fungus, *Magnaporthe oryzae*. Mihwa Yi and Yong-Hwan Lee. Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea

Heat shock protein 70 (Hsp70) family of genes are highly conserved through out the organisms including plants, animals, and fungi. The functions of any Hsp70 proteins in the rice blast fungus, *Magnaporthe oryzae*, however, have not been elucidated yet. The one matching to the yeast LHS1 (Lumenal Hsp Seventy) of the homologues in *M. oryzae* was deleted by targeted fungal transformation via *Agrobacterium tumefaciens*-mediated transformation. The vegetative growth of the mutants was slightly delayed and the pigmentation on the medium supplemented with manganese was reduced. Germination and appressorium formation of the *molhs1* mutants were not different from those of the wild type strain. However, both penetration and invasive growth stages were defective, which results in significant reduction on the pathogenicity of the fungus. Furthermore, the asexual reproduction was largely decreased in the *molhs1* compared to the wild type strain. A single ectopic insertion of partial fosmid clone including the full ORF of *MoLHS1* recovered the defective phenotypes on the *molhs1* mutant. The transcriptional expression of *MoLHS1* was specifically induced by endoplasmic reticulum stress conditions such as dithiothreitol or tunicamycin treatment, which shows the possibility that MoLHS1 might function as chaperone under ER stress like LHS1 in yeast. Here we report novel functions of Hsp70 gene in *M. oryzae* as an important player on the pathogenicity, mycelial growth, and the sporulation development.

446. Degradation of plant cell wall polysaccharides in relation to the infection process of *Magnaporthe grisea* on cereals. Ronald P. de Vries[1], Charissa de Bekker[1], Arnaud Lagorce[2], Cecile Ribot[3], Marc-Henri Lebrun[3]. [1]Microbiology, Utrecht University, Utrecht, The Netherlands; [2]Bayer Cropscience, Lyon, France; [3]UMR 2847 CNRS-Bayer Cropscience, Lyon, France.

The *Magnaporthe grisea* species complex attacks a wide range of grasses, including wheat, barley or rice, and is a model organism for studying plant fungal interactions. The early stage of the infection process is characterized by the formation of an appressorium that mediates the penetration of the fungus into host plant tissues through the puncture of cuticle and plant cell wall. Although this penetration process is mainly mechanical, requiring a high turgor in the appressorium, it can not be excluded that cell wall degrading enzymes (CWDE) could play a role in this process and in subsequent fungal spreading into plant tissues Analysis of *M. grisea* genome revealed a large number of genes encoding plant polysaccharides degrading enzymes. The set of *M. grisea* CWDE encoding genes was compared to the repertoire of *Aspergillus nidulans* CWDE, highlighting evolutionary trends specific of M. grisea that suggest an adaptation of *M. grisea* to its host plants. Analysis of the expression of these genes in rice leaves infected by *M. grisea* was performed using DNA micro array and real time RT-PCR demonstrating that some of these genes are highly expressed during the infection.

447. Molecular cloning of *Avr-Pia*, the avirulence gene in *Magnaporthe oryzae* toward the rice blast resistance gene *Pi-a*. Teruo Sone[1], Shinsuke Miki[1], Kotaro Matsui[1], Taketo Ashizawa[2], Hideki Kito[3], Kazuyuki Hirayae[2], and Fusao Tomita[4]. [1] Hokkaido Univ., Sapporo Japan, [2] Nat. Agric. Res. Cen., Niigata Japan [3]Nat. Inst. Agric. Sci. Tsukuba Japan, [4]Univ. of Air, Sapporo Japan

Avirulence genes in the genome of fungal plant pathogens are the main factor for the determination of their host (cultivar) specificity. We aimed to clone and analyze the avirlulence gene from the Japanese field isolates of *M. oryzae*. First, a mutant of strain Ina168, named Ina168m95-1, which gained the virulence toward rice cultivar Aichi- asahi with resistance gene *Pi-a*, was isolated. RAPD primers were screened for polymorphic amplification between the mutant and the parent. One primer, named OPM-1 resulted a DNA (named PM1) fragment absent in the mutant. Three cosmid clones including PM1 flanking region were screened from genomeic DNA library. One of these clones named 46F3 could complement the mutant phenotype, and thus strongly indicated that this clone contains the avirulence gene *Avr-pia*. The clone 46F3 contained many fragments of transposable elements. We divided 46F3 insert into six fragments, I to VI, that does not contain transposable elements, and individually cloned into hygromycin-resistant vector for the transformation of the mutant Ina168m95-1. The inoculation assay of transformants revealed that fragment V (3.5 kb) contains *Avr-pia*. This was also confirmed by PCR amplification assay of fragment V from field isolates. From isolates that infect *Pi-a*, no amplification was detected. Deletion mutants of the fragment V were used for the further focusing of *Avr-pia* locus. Finally we found an 1199 bp-DNA region responsible for *Avr-pia*, which includes a 255 bp-ORF with weak homology to bacterial cytochrome-like protein.

448. The MAD1 Adhesin of *Metarhizium anisopliae* Links Adhesion to Insect Cuticle with Blastospore Production and Virulence; A Second Adhesin (MAD2) Enables Attachment to Plant Surfaces. Chengshu Wang and Raymond J. St. Leger. Department of Entomology, University of Maryland, College Park, MD 20742, USA. stleger@umd.edu.

***Metarhizium anisopliae* is a fungus of considerable metabolic and ecological versatility, being a potent insect pathogen that unusually can also colonize plant roots. The mechanistic details of these interactions are unresolved. We provide evidence**

that *M. anisopliae* adheres to insects and plants using two different proteins, MAD1 and MAD2 that are differentially induced in insect hemolymph and plant root exudates, respectively, and produce regional localization of adhesive conidial surfaces. Expression of Mad1 in *Saccharomyces* allowed this yeast to adhere to insect cuticle. Expression of Mad2 caused yeast cells to adhere to a plant surface. Our study demonstrated that as well as allowing adhesion to insects, MAD1 at the surface of *M. anisopliae* conidia or blastospores is required to orientate the cytoskeleton and stimulate expression of genes involved in the cell cycle. Consequently, disruption of Mad1 in *M. anisopliae* delayed germination, suppressed blastospore formation and greatly reduced virulence to caterpillars. Disruption of Mad2 blocked adhesion of *M. anisopliae* to plant epidermis, but had no effects on fungal differentiation and entomopathogenicity. Thus regulation, localization and specificity control the functional distinction between Mad1 and Mad2, and enable *M. anisopliae* cells to adapt their adhesive properties to different habitats.

449. Expressing an insect-specific scorpion neurotoxin makes *Metarhizium anisopliae* hypervirulent to mosquitoes and caterpillars. Chengshu Wang and Raymond J. St. Leger. Department of Entomology, University of Maryland, College Park, MD 20742. stleger@umd.edu.

Insects cause $244 billion in damage to world agriculture every year and mosquito borne diseases are among the most devastating in modern times. *Metarhizium anisopliae* is being developed as a control agent against mosquitoes and other major pests, but its full potential has not been approached because of slow kill. Here we tested the ability of the promoter of the *Metarhizium anisopliae* hemolymph-induced collagen-like (Mcl1) gene to specifically target production of toxins into the hemocoel of infected insects. We expressed a codon-optimized synthetic gene encoding N- terminal secretary signals and an insect-specific neurotoxin (AaIT) from the scorpion *Androctonus australis*. The synthetic gene was introduced into a broad host range strain of *M. anisopliae*. Stable incorporation was confirmed by PCR and rapid hemolymph-specific expression confirmed by RT-PCR and assay of biologically active toxin. Improvements in pathogenicity were dramatic. Virulence was increased 22-fold against tobacco hornworm (*Manduca sexta*) caterpillars and nine-fold against adult yellow fever mosquitoes (*Aedes aegypti*), and was more rapidly effected. Furthermore, there were useful pre-lethal effects including reduced mobility and feeding as compared to hosts infected by the wild type. This work represents a significant step towards the development of hypervirulent insect pathogens for effective pest control.

450. Transposon tagging in *Fusarium culmorum*, a pathogen on cereals. M. Dufresne[1], V. Balmas[2], G. Ortu[1], M.-J. Daboussi[1] and Q. Migheli[2]. [1] Institut de Génétique et Microbiologie, Université Paris-Sud, Orsay, France [2] Dipartimento di Protezione delle Piante, Università degli Studi di Sassari, Italy

Crown and foot rot of wheat is an important soilborne disease caused by several species of filamentous fungi. *Fusarium culmorum* (W.G. Smith) Sacc. is one of the most common incitants of this disease worldwide and is able to produce type B trichothecenes. Aiming at deciphering mechanisms involved in pathogenicity and mycotoxin production of *F. culmorum*, we have recently started two transposon-based mutagenesis to identify genes governing these characters without a priori knowledge on their function. We first used the impala element, which was already shown to transpose in a wide range of Ascomycete species. A collection of 300 revertant strains has been generated and tested for pathogenicity on wheat under glasshouse condition. Following two rounds of wheat assays, 7 strains, more or less altered in pathogenicity have been identified among 175 strains with a reinsertion event. To date, one strain has been characterised in detail. Loss of pathogenicity results from the insertion of impala in the close 5' region of a gene encoding a putative HMG Co-A reductase, a function already described as involved in *F. graminearum* pathogenicity (Seong et al. 2006). In parallel, a systematic recovery of impala insertion sites has been started in order to determine its insertional preference. More recently, we introduced the double component mimp/impala to compare the tagging efficiency of both systems. Seong K et al.(2006) FGB 43:34-41.

451. Multiplication of the *PEP*-cluster-carrying conditionally dispensable chromosome by protoplast fusion in *Nectria haematococca* MPVI. Hamid Sadeghi Garmaroodi and Masatoki Taga. Department of Biology, Okayama University, Okayama 700-8530, Japan

Since the conditionally dispensable chromosomes (CDCs) in *N. haematococca* MPVI are known to be responsible for the higher fitness to the host plants of the strains harboring them, it is intriguing to examine the possibility of accumulation of CDC in the genome and its effect on the fitness of the fungus. In this study, we used protoplast fusion for producing strains with multiple copies of *PDA1*-CDC and analyzed the pathogenicity and homoserine (HS)- utilizing ability which are correlated with CDC. As the parents for electro- and PEG-mediated fusion, a lab strain bearing a single CDC marked with hygromycin resistance gene, Tr18.5 (Wassman and Van Etten, 1996), and a geneticin resistant transformant of the same strain were used. Using PCR, pulsed field gel electrophoresis and chromosome painting of fluorescence *in situ* hybridization, we detected at least two CDCs in the fusants. Cytological chromosome counting by fluorescence microscopy in parents and selected fusants showed that extra set(s) of A chromosomes in the fused protoplasts should

have been deleted during the regeneration of the fused protoplasts to single-spored cultures. All fusants with multiple copies of CDCs in their genomes showed higher virulence to pea and ability to use HS in the media than the parents.

452. Protein arrays for transcription factors analysis in *Magnaporthe grisea.* Malali Gowda [1], Jixin Deng[1], Yeon Yee Oh[1], Hee-Sool Rho[2], Cristiano Nunes[1], Jin-Rong Xu[3], Heng Zhu[2], Thomas Mitchell[1] and Ralph Dean[1]. [1]Fungal Genomics Laboratory, North Carolina State University, Raleigh, NC 27606, USA. [2]Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA. [3]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA.

***Magnaporthe grisea* is widely considered as a model fungus to study plant-fungal interactions. The signaling network in *M. grisea* is well established, which includes cAMP, MAP kinase and Ca/calmodulin pathways. However, understanding of downstream transcription factors that regulate the expression of these pathways is lacking. Through the whole genome annotation process, we have identified nearly 500 putative transcription factors in *M. grisea*, of which, 74 genes were found to have no intronic sequences in the coding regions. We further continued manual annotation and confirmed the expression of > 90% of these using expression data from EST, RL-SAGE and MPSS. We have cloned >80% of transcription factors from *M. grisea* into Gateway expression vectors, and are presently over expressing each in yeast to print *Magnaporthe* transcription factors array. These protein arrays will be used to assay the kinase specificity activity of putative transcription factors. Homologous gene-knockout and over-expression strategies will be deployed to map the transcription factor binding motifs and subsequently identify genes regulated by transcription factors using ChIP-chip hybridization and 454 sequencing approaches. We will present results from annotation and cloning efforts as well as preliminary phosphorylation assay.**

453. The role of high affinity iron uptake systems in *Ustilago maydis.* Britta Winterberg[1], Jan Schirawski[1], Franziska Leßing[1], Heiko Eichhorn[1], Uwe Linne[2] and Regine Kahmann[1]. [1] Max Planck Institute for Terrestrial Microbiology, D-35043 Marburg, Germany [2] Philipps-University, Department of Biochemistry, D-35043 Marburg, Germany

The phytopathogenic basidiomycete *Ustilago maydis* induces tumors on maize. Within these tumors the diploid fungal spores develop. *U. maydis* has two high affinity iron uptake systems. The permease-based system is necessary for full virulence of *U. maydis* on its host plant maize. In addition, *U. maydis* is able assimilate iron via the siderophores ferrichrome and ferrichrome A. Using microarrays we identified genes that are regulated both by iron and the iron responsive transcription factor Urbs1. We investigated the putative function of these genes in siderophore biosynthesis. We constructed deletion mutants of *sid2* and *fer3*, encoding two non-ribosomal peptide synthetases (NRPS), and *fer4*, *fer5*, and *fer8*, encoding a enoyl-CoA-isomerase, an acetylase and an oxidoreductase, respectively. HPLC analysis of culture supernatants revealed that the *sid2* deletion mutant only produced ferrichrome A, while the other mutants solely synthesized ferrichrome. Based on predicted function of proteins involved in ferrichrome A synthesis we postulated a biosynthetic pathway that is currently under investigation. To analyze the role of siderophores during pathogenic development, plant infection assays with double deletion mutants of the NRPS Sid2 and Fer3 were performed. These mutants were as pathogenic as the wild type and produced viable spores. However, spore progeny were diploid indicating a meiosis defect. Additionally, double deletion mutants of *sid2* and *fer3* mutants are unable to grow on low-iron medium, suggesting that, in contrast to the permease-based system that is crucial for biotrophic development, the siderophore-based system is essential for germination and growth outside the plant.

454. A STE12-like transcription factor from the plant pathogen *Colletotrichum lindemuthianum* regulates adhesion and expression of a extracellular proteins. Bernard Dumas[1], Joanne Wong Sak Hoi[1], Nafees Bacha[1], Claude Lafitte[1], Gisèle Borderies[2], Michel Rossignol[2], Pierre Rougé[1]. [1]UMR 5546 CNRS-Université Paul Sabatier Toulouse III, Castanet-Tolosan, France [2]Plateforme protéomique de Toulouse, site IFR40, Castanet-Tolosan, France

In phytopathogenic fungi, STE12-like genes encode transcription factors essential for appressorium-mediated host penetration. However, their downstream targets are still largely unknown. We recently identified regulatory elements similar to those recognized by Ste12p in the promoter of a polygalacturonase gene in the bean pathogen *Colletotrichum lindemuthianum*, suggesting that STE12-like transcription factors could be involved in the regulation of genes coding cell wall degrading enzymes. This finding led us to isolate a STE12-like gene from *C. lindemuthianum* (CLSTE12) and to produce disruption mutants. Phenotypic analyses of the mutants revealed reduced pectinase activity and conidial adhesion to an artificial surface. Analysis of cell surface proteins allowed the identification of a major protein, Clsp1p (*Colletrichum lindemuthianum* Surface Protein 1), which was absent from the mutants. Accordingly, CLSP1 transcript accumulation was dramatically reduced in the clste12 mutants. Clsp1p belongs to a new family of fungal wall-associated proteins putatively involved in the interaction of fungal cells with abiotic or biotic surfaces.

455. Annotation of signaling pathways genes families in the ectomycorrhizal basidiomycete *Laccaria bicolor* reveals an expansion of a G-proteins encoding genes family. Sebastien Duplessis[1], Laccaria genome consortium & DOE Joint

Genome Institute[2]. [1]UMR 1136 INRA/UHP Nancy 1 Interactions Arbres/Micro-organismes INRA Nancy, 54280 Champenoux France. [2]DOE Joint Genome Institute Walnut Creek CA 94598 USA.

We have investigated the signaling pathways genes families in the genome sequence of the ectomycorrhizal basidiomycete *L. bicolor*. We focused on the known components of the G-protein mediated signaling pathways (i.e., G-proteins, GPCRs, PKAs, Adenylate cyclases) and MAP kinases that were previously described in other filamentous fungi. Our results show a large expansion of the gene family coding for heterotrimeric G-protein alpha subunits in *L. bicolor* with 39 putative members compared to other filamentous ascomycetes or basidiomycetes where only 3 to 4 copies had been described so far. Homologs of fungal G-alpha proteins known for their role in filamentous growth or mating were found in *L. bicolor* while no homolog of G-alpha related to virulence in phytopathogenic fungi was detected. In addition, two new classes of G-alpha were observed: one corresponding to shorter proteins and another one with similarities to G- alpha sequences described in *Ustilago maydis* (UmGPA4) and *Aspergillus oryzae* (AoGaoC). These new classes of G-alpha proteins supported by ESTs and expression profiling, may participate to fine-tune molecular crosstalk between the ectomycorrhizal fungus and tree roots during symbiosis development.

456. Genomic approaches to unravel *Aphanomyces euteiches* plant Interactions. Elodie Gaulin, Amin Madoui, Arnaud Bottin, Christophe Jacquet, Bernard Dumas. UMR CNRS-Université Paul Sabatier, Toulouse III, 24 Chemin de Borde-Rouge, 31326 Castanet-Tolosan, France, gaulin@scsv.ups-tlse.fr

***Aphanomyces* sp. (*Saprolegniaceae*) houses plant, animal and pathogens found in both terrestrial and aquatic habitats. *Aphanomyces euteiches* Drechs. causes seedling and root rot diseases on legumes and is the most pea (*Pisum sativum*) serious disease in several countries. Because the strictly soil borne pathogen may survive for long periods in the soil (more than 10 years), the only existing control is to avoid cultivating peas in infested field for many years. While a huge genomic research effort were devoted to Oomycete such as *Phytophthora, Aphanomyces* received little attention and the mechanism by which it infects its hosts is largely unknown. *A. euteiches* is an interesting model to study plant-microbe interaction since it display differential specificity of interaction with the legume model *Medicago truncatula* . To gain a first insight into the transcriptome of *A. euteiches* a genomic approach have been undertaken. A total of >20000 EST were generated from 2 cDNA libraries. 7 000 consensus sequences were assembled and will be available through a public database. Annotation of ESTs revealed a number of genes that could function in virulence. Progress in this research will be presented.**

457. *MoRAC1* , a *Magnaporthe oryzae* Rho GTPase gene, is required for conidial morphogenesis and pathogenicity. Sung-Yong Yoo, Maruthachalam Karunakaran, and Yong-Hwan Lee. Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea.

Rac proteins, highly conserved members of the Rho family GTPase, play important roles in control of polarized growth in many organisms but the specific function of these proteins in plant pathogenic fungi is yet to be explored. During large scale insertional mutagenesis through *Agrobacterium tumefaciens*-mediated transformation in the rice blast fungus *Magnaporthe oryzae*, we have identified a transformant where T-DNA was inserted in the gene, *MoRAC1*, encoding Rho type GTPase. Phenotype of the *morac1* mutants exhibited pleiotropic effects on conidial development such as reduced conidiation, deformation in conidia and conidiophore development. The T-DNA insertion mutant phenotype was confirmed when creating null mutant for the *MoRAC1* gene. *morac1* mutants displayed defects in polarized growth and cell differentiation similar to T-DNA insertion mutant. Furthermore, *morac1* mutant is also defective in appressorium formation and pathogenicity. Genetic complementation using the ORF of the *MoRAC1* from fosmid clone fully recovered all defective phenotypes. Whole genome microarray analysis of *morac1* suggests that nearly hundreds of the genes were regulated more than two folds up and down, strikingly some of the genes are highly associated with surface recognition, germtube and hyphal formation, conidiation, pathogenicity, and recycling of cell wall compounds. These results demonstrate that *MoRAC1* is a crucial player for conidial morphogenesis, cell growth, and pathogenicity in *M. oryzae*.

458. *Agrobacterium tumefaciens*-mediated transformation (ATMT) in two *Colletotrichum* Species; *C. falcatum* and *C. acutatum*. Maruthachalam Karunakaran[1], Hee-Sool Rho[1], Soonok Kim[1], N. Vijayan Nair[2] and Yong-Hwan Lee[1]. [1]Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea. [2]Biotechnology Laboratory, Sugarcane Breeding Institute, Coimbatore 641-007, India.

***Agrobacterium tumefaciens*-mediated transformation is becoming an effective system as an insertional mutagenesis tool in filamentous fungi. In this study, ATMT was developed and optimized for two *Colletotrichum* species; *C. falcatum* and *C. acutatum*, the causative agents of red rot of sugarcane and anthracnose of pepper respectively. *A. tumefaciens* strain pYL63e, carrying hygromycin phosphotransferase gene (*hph*) and green fluorescent protein gene, was used to transform the conidia of these two *Colletotrichum* species. The transformation efficiency was higher in *C. falcatum* than that of *C. acutatum*, and it was also correlated with the co-cultivation time and bacterial cell concentration. The presence of *hph* and**

GFP genes were verified by PCR and microscopic analysis. About 70% of the transformants had a single copy of the T-DNA insertion in both *C. falcatum* and *C. acutatum*. Southern hybridization indicated that T-DNA integrated randomly in both the fungal genome. The T-DNA flanking regions were identified in transformants through thermal asymmetrical interlaced PCR followed by sequencing. Our results suggest that ATMT can be used as a molecular tool to identify and characterize pathogenicity related genes of these two important *Colletotrichum* species.

459. Hexosaminidase and plant growth promotion by *Trichoderma hamatum*. Lauren S. Ryder and Christopher R. Thornton. School of Biosciences, University of Exeter, Washington Singer Labs., Perry Eoad, Exeter, UK, EX4 4QG

Trichoderma species are important agents in the biocontrol of root pathogens and are attractive alternatives to synthetic pesticides. In addition to biocontrol properties, certain strains display plant-growth-promotional activities. While the involvement of chitinases in the biocontrol activities of Trichoderma species has been studied in depth, little attention has been paid to the role of chitinases in the 'nutritional chitinolysis' of Trichoderma spp. A rhizosphere-competent strain of T. hamatum (strain GD12) has been identified that elicits a 5-fold increase in the root and photosynthetic biomass of A. thaliana and other plant species during growth in low nutrient status plant growing media such as peat. High levels of hexosaminidase activities occur during saprotrophic colonization of peat by GD12 suggesting that the fungus is mobilizing hexosamines present in the medium. Consequently, we set out to determine the role that hexosaminidases play in saprotrophism of the fungus and whether mobilization of hexosamines could account for the plant-growth-promotion witnessed during Trichoderma-plant interactions. A 1.9kb fragment of the extracellular enzyme hexosaminidase was identified using primers based on the T. harzianum exc2y sequence. Strains disrupted in hexosaminidase production were developed using an insertional mutagenesis strategy and preliminary phenotypic analysis shows that mutants display enhanced plant growth-promotion activities. The mechanism for this phenomenon is currently being determined.

460. Signaling for symbiosis and pathogenicity. Natalia Requena, Stephanie Heupel and Hannah Kuhn. Institute for Applied Microbiology: Plant-Fungal Interactions group, University of Karlsruhe, Hertzstrasse 16, 76187 Karlsruhe, Germany; natalia.requena@bio.uka.de

Plants have evolved to interact with different microorganisms and establish with them different types of associations ranging from parasitic to mutualistic. In doing so, the immune system of the plant has developed to recognize signal/pattern molecules from those microorganisms to discriminate between deleterious and beneficial partners. The establishment of the arbuscular mycorrhiza symbiosis belongs to the second group and plants forming this type of symbiosis, most vascular plants, recognize specifically their symbiotic partners modifying in consequence their genetic programs to accommodate them. The molecular bases of this recognition process are starting to be understood and point to common signaling pathways shared with other microbe-plant associations like the nitrogen fixation symbiosis or pathogenic interactions and to specific signaling pathways only but commonly elicited by all arbuscular mycorrhizal fungi. In searching for fungal proteins involved in this early recognition process we identified in the AM fungus *Glomus intraradices* a novel protein Gin1 with a two domain structure. We have recently identified that this protein is post-translationally modified by plant effectors to render an amino-terminus with a lipid moiety covalently bound. Interestingly, a putative orthologue of the amino-terminus exists in a few fungi including the plant pathogens *Magnaporthe grisea* and *Giberella zeae*. In order to identify whether this common protein plays a dual role in symbiotic and pathogenic interactions we have taken *M. grisea* as a model and studied the KO phenotype during plant infection and the effect of its replacement with the mycorrhizal fungal protein. (This project is financed by the DFG 1556/1-3; DFG 1556/4-1).

461. Differential gene expression between the biotrophic-like and saprotrophic mycelia of the Witches' broom pathogen *Moniliophthora perniciosa.* Johana Rincones[1], Leandra Scarpari[1], Jorge Mondego[1], Maricene Sabha[1], Marcelo Carazzolle[1], Joan Barau[1], Lyndel Meinhardt[2], and Gonçalo Pereira[1]. [1]Lab. Genômica e Expressão, IB, UNICAMP, Campinas/SP, Brazil. [2] Sustainable Perennial Crops Laboratory, USDA–ARS, Beltsville/MD, USA. johana@lge.ibi.unicamp.br

In order to improve our understanding of the molecular mechanisms involved in the biotrophic development of *M. perniciosa*, we performed DNA microarrays and EST analyses of the biotrophic and saprotrophic stages of this fungus, grown *in vitro*. Reads from 3 cDNAs libraries (glucose- and cacao-induced saprotrophic mycelia and cacao-induced biotrophic mycelium) were clusterized into 1596 unigenes. DNA microarray analysis was performed for 2304 genomic DNA fragments, with probes prepared from mRNA of the biotrophic and saprotrophic mycelia. Our results suggest that both types of mycelia show different carbon metabolisms, with glycolysis and the glyoxalate shunt being repressed in the biotrophic phase. Moreover, the biotrophic stage showed induced transcripts indicative of amino acid starvation, as well as several characterized as pathogenicity factors, such as Glyoxal oxidase, ceratoplatanin, proteases and peroxidases. Differential expression of selected genes was confirmed by Real Time-PCR. This work is the first to analyze global gene expression between the infective and saprotrophic stages of a hemibiotrophic fungus. Support: FAPESP and CNPq.

462. Towards a transformation system for the arbuscular mycorrhizal model fungus *Glomus intraradices.* Nicole Helber and Natalia Requena, Plant-Fungal Interactions Group, Institute of applied Biosciences University of Karlsruhe, Germany

Arbuscular mycorrhiza (AM) is known as one of the evolutionary oldest plant symbiosis. However, the knowledge about the biology of AM fungi has remained relatively unstudied to date. This is due to their obligate symbiotic and biotrophic lifestyle. Furthermore, spores and hyphae are multinucleated which complicates their genetic analysis. To advance our understanding of symbiosis establishment and functioning we are trying to establish a transformation method for the model AM fungus *Glomus intraradices*. Therefore we are using *Agrobacterium*-mediated transformation and biolistic transformation. As target tissue we are using ungerminated or germinated spores. Initially we used GFP as marker to monitor transformants. However, because of the strong autofluorescence of the fungal tissue in the green channel we have constructed new vectors containing the dsRed gene fused to the NLS sequence of stuA from *Aspergillus nidulans*. The reporter gene is driven by the *Glomus mosseae* promoters GmPMA1 or Gmfox2. In control experiments we could show that Gmfox2 promoter is active in *Aspergillus nidulans*. First data using the biolistic approach in*G. intraradices* showed red fluorescence spots in some spores and hyphae. Although we have not proven yet that these red spots are located in the nuclei we regard this as a good starting-point for further investigations.

463. Silencing of *Kex2* significantly diminishes the virulence of *C. parasitica*. Debora Jacob-Wilk[1], Massimo Turina[1,2], Pam Kazmierczak[1] and Neal Van Alfen[1]. Department of Plant Pathology, University of California, Davis. [1] Department of Plant Pathology, University of California, Davis, CA. 95616, USA. [2] Current address: Istituto di Virologia Vegetale, CNR Torino, Italy.

***Cryphonectria parasitica* is the causal agent of chestnut blight, its infection with *Cryphonectria hypovirus 1* (CHV1) results in hypovirulent strains of the fungus. The discovery that several of the CHV1 down regulated genes encoded for secreted proteins containing the Kex2 processing consensus in conjunction with the recent finding that CHV1 utilizes fungal vesicles for replication that co-localize with *trans*-Golgi network marker Kex2, led us to investigate the possible role of Kex2 in fungal pathogenicity. We report on the cloning and analysis of *Cryphonectria parasitica Kex2* gene. *C. parasitica Kex2* gene sequence analysis showed high homology and structural similarities to other kexin like proteins from different Ascomycetes. In order to assess *cpKex2* possible role in fungal virulence, antibodies were raised and Kex2 silenced mutants were generated. Southern blot analyses of *cpKex2* showed a single copy gene while Western blots showed Kex2 expression at every time point checked. Although growth rate was not considerably affected by silencing, silenced isolates had significantly reduced virulence and this reduced virulence was in correlation with Kex2 levels of enzymatic activity and relative mRNA transcript levels as measured by real time RT-PCR.**

464. Penetration Resistance: The First Line of Defense Against Invasive Pathogens. Shauna Somerville, Monica Stein, Matt Humphry, Laurent Zimmerli, Melisa Lim. Carnegie Institute, Stanford, CA, 94305, U.S.A. SSomerville@stanford.edu

A majority of host plants are resistant to the majority of pathogens in their environment. The underlying mechanisms of this form of disease resistance are poorly studied. The Paul Schulze-Lefert lab (Cologne, Germany), Hans Thordal-Christensen (Copenhagen, Denmark) lab and our lab, working collaboratively, identified mutants of the model plant *Arabidopsis thaliana* that were compromised in their resistance to the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*, which is pathogenic only on barley. Several Arabidopsis genes, not previously implicated in plant resistance mechanisms, have been identified by this approach, including *PEN1 (=SYP121)* a syntaxin and functional homologue of the barley *ROR2* gene; *PEN2*, a glycosyl hydrolase; and *PEN3 (=PDR8)*, an ABC transporter. In addition, *MLO2* was identified as the functional homologue of the barley *MLO* gene, which confers penetration resistance when inactivated. The plasma membrane localized MLO, PEN1 and PEN3 proteins accumulate locally at penetration sites in inoculated plant tissues. These observations suggest that plants mount effective, broad spectrum defenses designed to block pathogen invasion across the host cell wall and entry into cells. Over evolutionary time, virulent powdery mildew species, such as *Golovinomyces cichoracearum*, a pathogen of Arabidopsis, must presumably have acquired the ability to avoid these defenses by stealth or to be insensitive to them.

465. Gene expression analyses of *Fusarium graminearum* during infection of wheat using microarrays and reporter gene constructs. Donald M. Gardiner[1], Amber E. Stephens[1,2], Anca Rusu[1], Jo K. Bursle[1], Agnieszka Mudge[1], Kemal Kazan[1], Alan L. Munn[2], John M. Manners[1]. [1]Commonwealth Scientific and Industrial Research Organisation (CSIRO), Plant Industry, 306 Carmody Rd, St Lucia 4072, Queensland, Australia [2]The Institute for Molecular Bioscience, The University of Queensland, St Lucia, 4072, Queensland, Australia Donald.Gardiner@csiro.au

Fusarium Head Blight is one of the most important diseases of wheat worldwide. In Australia, *F. graminearum* and the closely related fungus *F. pseudograminearum*, cause sporadic head blight epidemics and are also responsible for crown rot

disease in wheat. These diseases can lead to substantial yield losses and accumulation of mycotoxins, particularly trichothecenes, which adversely affect human and animal health when consumed. We are using green fluorescent protein reporter constructs to investigate the temporal and spatial expression patterns of a number of candidate genes potentially involved in the infection process, particularly at the stem base. Candidates include the Tri5 trichothecene biosynthesis gene and a number of genes identified as being expressed in the early stages of crown rot disease using the *F. graminearum* microarrays and RT-qPCR.

466. A multi-locus sequence typing system for the chytrid, *Batrachochytrium dendrobatidis.* Russell Poulter, Margi Butler, Tim Goodwin. University of Otago, New Zealand. russell.poulter@stonebow.otago.ac.nz

***Batrachochytrium dendrobatidis* is a recently emerged pathogen of amphibia responsible for a world-wide pandemic, leading to numerous species extinctions. All isolates of *B. dendrobatidis* have very similar genomes and this restricts phylogenetic analyses and epidemiological studies. We will describe a multi-locus sequence typing system (MLST) based on the several inteins we have detected in the genome of this species. Intein encoding sequences are typically less constrained than most coding sequences and they are therefore well suited to analysing the phylogenies of homogeneous species. Inteins are proteins that are removed post-translationally from a host protein. This is an autocatalytic splicing process necessary for the maturation of the host protein. Inteins typically contain not only the necessary protein splicing domains but also a homing endonuclease (HEG). The HEG enables the intein encoding sequence to spread through the gene pool of a species and potentially to also undergo horizontal transmission to other species. The HEG activity is therefore seldom used and the HEG could be completely inactive without adversely affecting the protein splicing function. This is the explanation of the relatively unconstrained sequence of inteins.**

467. Characterization of Yeasts Associated with Green Tea Leaves. Pham Tran To Nhu, Do Quyen, Ngo Duc Duy, Hoang Quoc Khanh. Institute of Tropical Biology, Viet Nam Academy of Science and Technology 1 Mac Dinh Chi St., Dist. 1, Ho Chi Minh City, VIETNAM

This paper is described yeast strains isolated from tea leaves collected in Bao Loc and Gia Lai provinces, Viet Nam. Five yeast strains of BL1, BL2, BL3, BL4 and GL were identified by using conventional (morphology, physiology, biochemistry) and molecular phylogenetic studies. The vegetative reproduction occurs in these strains by budding. Lipase and acid production from glucose were negative. Fermentation of sugar such as glucose, galactose, and maltose were positive. All strains revealed the capacity of gelatin liquefaction, except BL2. These strains assimilated carbon compounds glucose, galactose, sucrose, L-sorbose, glycerol, sorbitolâ€| and nitrogen compounds (NH4)2SO4, ethylamine, L-lysine. Based on the sequence analysis of the 26S-rDNA D1/D2 domains and internal transcribed spacer (ITS) regions, then compared with the databases of the Genbank NCBI, the results show that BL1 and GL belonged to the genus *Candida*, near *Candida tropicalis* and *Candida fermentati* respectively. Two strains of BL2 and BL4 were known as *Rhodotorula mucilaginosa* and *Rhodotorula sp.* CBS 8885. Finally, BL3 was the nearest *Rhodosporidium fluviable*. These strains (BL2, BL3 and BL4) were further investigated the production of carotenoid.

468. Signaling in *Botrytis cinerea* – a focus on small GTPases and early steps of infection. Leonie B. Kokkelink and Paul Tudzynski. Westfaelische Wilhelms University, Institute of Botany, Schlossgarten 3, D-48149 Muenster, Germany

The necrotrophic ascomycete *B. cinerea* causes gray mold diseases on various crop plants. Up to the present, several components of signaling pathways were identified that are involved in pathogenicity. So far there is little knowledge about the impact of small GTPases belonging to the Ras superfamily on pathogenicity in *B. cinerea* and about connections to other signaling pathways. We are currently investigating several small GTPase encoding genes from *B. cinerea*: two genes encoding Rho type GTPases, *bccdc42* and *bcrac*, and two Ras homologues, *bcras1* and *bcras2*. By different approaches like pathogenicity assays, epidermis penetration experiments, germination assays and microscopy we determine the role of the small GTPase deletion strains in pathogenicity and early infecton stages. *bcras1* and *bcrac* deletion mutants are viable, but show a severe phenotype characterized by an undirected hyphal growth with abnormal branching pattern, a lack of sporulation and impaired infection ability. In contrast, the Δ*bccdc42* mutants grow properly on rich media and are able to form conidia. This strain is able to infect plants, though the infection progress is delayed. Furthermore, we are interested in the interconnections and hierarchies to other major signaling pathways (e. g. cAMP signaling / MAP kinases). For instance, phosphorylation experiments are carried out to elucidate the connection between Ras proteins and MAPK.

469. A secondary metabolite is involved in recognition of the blast fungus Magnaporthe grisea by resistant rice cultivars. Jérôme Collemare[1], Sabrina Bouras[1], Heidi U. Böhnert[1], Isabelle Fudal[1], Zhongshu Song[2], Russell J. Cox[2], Didier Tharreau[3] and Marc-Henri Lebrun[1]. [1]UMR2847 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]School of Chemistry, University of Bristol, Cantock's close, Clifton, Bristol BS8 1TS, UK. [3]UMR BGPI, CIRAD-INRA-AGROM, TA41/K, 34398 Montpellier Cedex 05, France

Recognition of the fungal plant pathogen *Magnaporthe grisea* by resistant rice cultivars is controlled by interactions between fungal avirulence genes (AVR) and their corresponding plant resistance genes (R). Most fungal AVR genes encode small peptides secreted into host tissues during infection. ACE1 from *M. grisea* differs from other AVR genes as it encodes a polyketide synthase fused to a non-ribosomal peptide synthetase, an enzyme involved in the biosynthesis of a secondary metabolite. This AVR gene controls the production of a signal recognized by rice cultivars carrying Pi33 resistance gene. ACE1 is specifically expressed in mature appressoria during penetration of the fungus into rice leaves. The protein Ace1 is only detected in the cytoplasm of appressoria and not in infectious hyphae differentiated inside infected epidermal cells. Ace1-ks0, a non-functional ACE1 allele obtained by site-directed mutagenesis of an amino acid from polyketide synthase KS domain essential for its enzymatic activity, is unable to confer avirulence. This result suggests that the avirulence signal recognized by Pi33 is not the Ace1 protein, but the secondary metabolite synthesized by Ace1. In order to characterize this metabolite, ACE1 was expressed in *M. grisea* under the control of a constitutive promoter. ACE1 was also expressed under the control of an inducible promoter in Aspergillus oryzae and in Fusarium venenatum. Secondary metabolites produced by these transgenic strains are currently analyzed by LC-MS-MS (coll. Certon, Metcalf and Drivon, Bayer CropScience, France). At the ACE1 locus, we identified 14 genes predicted to encode enzymes involved in secondary metabolism, including two enoyl-reductases and a binuclear zinc-finger transcription factor. These genes have the same expression pattern as ACE1 defining a cluster of co-expressed genes, suggesting that they are involved in the same biosynthetic pathway. The inactivation of these genes in an avirulent isolate is underway to assess their role in the biosynthesis of the metabolite recognized by Pi33 resistant rice cultivars.

470. Functional analysis of *Botrytis cinerea* NEP-like proteins. Yaite Cuesta Arenas, Alexander Schouten, Peter Vredenbregt, Mirjam Dieho , Beatrice Uwumukiza & Jan van Kan. Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands

Necrosis and ethylene inducing proteins (NEP) have been described in bacteria, oomycetes and fungi and have been proposed to act as phytotoxin in dicotyledons but not in monocotyledons. However, the mechanism of action is not known. *Botrytis cinerea* contains two NEP-like proteins, BcNEP1 and BcNEP2, which have been produced in *Pichia pastoris*.Infiltration of purified proteins into *Nicotiana benthamiana* leads to induction of ethylene in a dose-dependent manner. BcNEP2 is less active in inducing ethylene than BcNEP1.BcNEP1 and BcNEP2 contain a number of cysteines that may form disulfide bridges, as well as several potential posttranslational modification motifs. In order to study which amino acid residues are important for phytotoxic activity, site directed mutagenesis was performed and mutant proteins were expressed transiently in *N. benthamiana* through *Agrobacterium tumefaciens* (ATTA). Also through this approach, GFP was coupled to the C-terminus of BcNEP1 protein. The fusion protein retained phytotoxic activity and will be used for localization studies.

471. Defense of *Aspergillus fumigatus* against reactive oxigen species mediated by *Afyap1*. Franziska Lessing, Olaf Kniemeyer and Axel A. Brakhage. Leibniz Institute for Natural Products and Infection Biology – Hans-Knoell-Institute, Friedrich Schiller University, Jena, Germany Contact: franziska.lessing@hki-jena.de

With the increasing number of immunocompromised individuals *Aspergillus fumigatus* has become one of the most important opportunistic fungal pathogens. During infection *A. fumigatus* is confronted with a number of defence mechanisms in the host, particulary neutrophiles and macrophages, which kill conidia by producing reactive oxygen species. We identified a homologe of the AP1 like transcription factor *Yap1* from yeast in *A. fumigatus* which we designated *Afyap1*. In yeast, Yap1p was found to be a global regulator for oxidative stress response and required for the protection of the cell against H_2O_2 and other reactive oxygen species. Yap1 is transported in and out the nucleus under nonstressed conditions. The nuclear export is inhibited by oxidative stress and Yap1 induces the transcription of target genes. Nuclear loaclisation of an Afyap1-eGFP fusion in *A. fumigatus* was dependent on the presence of H_2O_2 and diamide. To identify new targets of *Afyap1*, we compared the proteome pattern from H_2O_2 induced and uninduced wild-type mycelia and a Afyap1 deletion strain by 2D-gel analysis.

472. Investigating the roles of reactive oxygen species during *Mycosphaerella graminicola* infection of wheat. Siân Deller, John Keon, John Antoniw, Kim Hammond-Kosack, and Jason Rudd.. Wheat Pathogenesis Programme, Plant- Pathogen Interactions Division, Rothamsted Research, Harpenden, Herts., AL5 2JQ, UK

***Mycosphaerella graminicola* (anamorph *Septoria tritici*) is a hemibiotrophic ascomycete pathogen of wheat leaves. Infection reduces crop yield via the appearance of chlorotic / necrotic lesions, which reduce the photosynthetically active leaf area. In the early stages of infection, following penetration through stomata, the fungus grows in the leaf intercellular space without producing any specialised feeding structures or causing visible disease symptoms. In the later infection stages, fungal biomass increases, hyphal nutrition becomes necrotrophic and localised host programmed cell death occurs (1). Septoria leaf blotch disease of wheat is currently regarded as the most economically damaging pathogen of wheat in the UK and Western Europe. Microscopy with reactive oxygen species (ROS)-specific stains has shown that hydrogen**

peroxide and superoxide are present in infected leaves, and that intensity of ROS stains increases with disease progression. In particular ROS are present on and within the asexual fruiting bodies, pycnidia. The involvement of ROS in the development of fungal sexual fruiting bodies has been reported (2, 3). Expression profiling of M. graminicola during infection of susceptible wheat genotypes has shown a cluster of genes, many ROS-associated, that have greatly increased expression as disease progresses and symptoms become visible. This project aims to better understand the molecular basis of the involvement of ROS in the disease-causing ability of *M. graminicola* on wheat. EST microarray datasets have been produced which have compared fungal gene expression under different growth conditions (4, 5). The microarray has also been employed to investigate to what extent application of exogenous ROS alone can induce gene expression patterns similar to those seen at the later stages of plant infection. Access to the sequenced genome allows targeted *Agrobacterium*-mediated gene deletion to be used for the investigation of fungal genes involved in the production of ROS or the oxidative stress response. Deletion analysis will allow comparison between transformant strains and wild-type IPO323 via *in vitro* and *in planta* characterisation and use of the EST microarray. 1.Keon, J., Antoniw, J., Carzaniga, R., Deller, S., Hammond-Kosack, K., & Rudd, J. (2007) *MPMI (In press)*. 2.Malagnac, F., Lalucque, H., Lepere, G., & Silar, P. (2004) *Fungal Genetics and Biology* 41, 982-997. 3.Lara-Ortiz, T., Riveros-Rosas, H., & Aguirre, J. (2003) *Molecular Microbiology* 50, 1241-1255. 4.Keon, J., Antoniw, J., Rudd, J., Skinner, W., Hargreaves, J., & Hammond-Kosack, K. (2005) *Fungal Genetics and Biology* 42, 376-389. 5.Keon, J., Rudd, J. J., Antoniw, J., Skinner, W., Hargreaves, J., & Hammond-Kosack, K. (2005) *Molecular Plant Pathology* 6, 527 - 540. This project receives financial support from Syngenta and the Biotechnology and Biosciences Research Council (BBSRC) of the UK. Rothamsted Research receives grant aided support from the BBSRC.

473. Expression of GFP in the obligate biotrophic oomycete *Plasmopara halstedii* using electroporation and a newly developed mechanical method (Löchern). Timo Hammer, Otmar Spring, Marco Thines. University of Hohenheim, Institute of Botany 210, 70593 Stuttgart, Germany, t-hammer@uni-hohenheim.de

Transformation of several oomycete species has been achieved during the past few years. Among these are plant pathogens of the genera *Pythium* and *Phytophthora*, which can be cultivated on media. In case of the obligate biotrophic pathogens belonging to the downy mildews (Peronosporaceae), no transgenic expression of any gene has been reported. In the model system *Plasmopara halstedii* and *Helianthus annuus* we have therefore tried several methods to obtain *Pl. halstedii* strains expressing GFP as a first starting point for further investigations into plant-pathogen interactions. Using electroporation and Löchern, a novel technique for transforming obligate biotrophic organisms we could achieve GFP expression in sporangia and sporangiophores of *Pl. halstedii*. Löchern involves injuring pathogen-infected leaves with a thin needle and at the same time applying a solution of the transformation vector on the leaf surface. Because of the easy handling and the possibility to circumvent the need for regeneration on media, Löchern could become an important technique in transforming obligate biotrophic pathogens.

474. Identification of virulence genes in *Fusarium oxysporum* by large-scale transposon tagging. M. Sánchez López-Berges[1], M. Dufresne[2], C. Hera[1], H. abd el Wahab[2], M.I.G. Roncero[1], M.J. Daboussi[2], A. Di Pietro[1]. [1]Departamento de Genética, Universidad de Córdoba, Campus Rabanales Ed. C5, 14071 Córdoba, Spain. E- mail: ge2snlpm@uco.es [2]Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France.

Forward genetic screens are efficient tools to dissect complex biological processes such as fungal pathogenicity. A transposon tagging system was developed in the vascular wilt fungus *Fusarium oxysporum* by inserting a novel modified version of the *impala* element upstream of the *Aspergillus nidulans niaD* gene, thus allowing easy monitoring of transposition events and isolation of revertants showing wild type growth on nitrate. In a pilot study, 1500 Nit^+ revertants were screened for altered virulence using a rapid infection assay on apple slices. Seven mutants exhibiting reduced virulence were identified and their phenotype was confirmed by infection assays on tomato plants. In 5 of them, the genomic region flanking the insertion site was characterized and the transposon insertion confirmed by Southern blot analysis. In 4 mutants, the insertion was found upstream of a putative ORF, whereas in the remaining mutant it was located within an ORF. Targeted knockout of the tagged genes is currently in progress to confirm their role in virulence.

475. Detoxyfication of progesterone in fungi *Fusarium oxysporum* and *Ustilago maydis* by progesterone-induced enzymes. Anna Poli, Helena Lenasi. University of Ljubljana, Faculty of Medicine, Institute of Biochemistry Ljubljana, Slovenia

Progesterone was previously shown to induce in filamentous fungi *Rhizopus nigricans* (phylum Zygomycota, class Zygomycetes, order Mucorales) and *Cochliobolus lunatus* (phylum Ascomycota, class Acsomycetes, order Plesporales) a multienzyme steroid hydroxylating system. It was also observed that progesterone signalling was mediated via receptors located in the plasma membrane and inside mycelia. It is generally believed that the induced enzyme system is involved in the detoxyfication process. In the present study we tried to answer the question whether this progesterone action is general in fungi. For our investigation we chose plant pathogens from phyla Ascomycota and Basidiomycota: *Fusarium oxysporum* (class Acsomycetes, order Hypocreales), *Ustilago maydis* (class Ustilaginomycetes, order Ustilaginales). In

liquid growth medium progesterone was applied. Steroids were extracted from mycelia and from the medium in different time intervals. The content of progesterone and progesterone derivatives were analysed either by TLC or HPLC and identified by gas chromatography/mass spetrometry. Fusarium oxysporum transformed progesterone mainly into 11-hydroxyprogesterone which appeared mostly in the medium, whereas Ustilago maydis transformed progesterone into a reduced product of A-steroid ring in addition to some hydroxylated products. The biological role of progesterone-induced enzymes is most likely transformation of toxic progesterone into less toxic products.

476. The *Phytophthora sojae* avirulence gene *Avr*1a, encoding an RxLR effector protein, displays copy number polymorphism. Dinah Qutob [1], Brett M. Tyler [2],and Mark Gijzen [1]. [1] Agriculture and Agri-Food Canada, London,ON, Canada. [2] Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA, USA.

We have completed the map-based cloning and identification of the *Phytophthora sojae* avirulence gene, *Avr*1a. Mapping of *Avr*1a required more than 1500 F_2 individuals from two separate crosses, illustrating the relatively low recombination frequency of the region. Problems in physical mapping occured even after completion of the *P. sojae* genome sequence, because the *Avr*1a gene does not occur on a large assembly contig. Physical mapping was aided by comparison to a syntenic region in *Phytophthora ramorum*. Eventually, we identified an open reading frame within the *Avr*1a delimited region in *P. sojae* that encodes a secreted RxLR-dEER protein of 121 amino acids. Transcriptional analysis demonstrated that the gene is expressed during infection, and that differences in the expression level could be correlated to *Avr*1a phenotype. Transient expression of this gene in soybean triggered an *Rps*1a -specific cell death response, confirming that it corresponds to *Avr*1a. Preliminary evidence suggests that multiple copies of *Avr*1a occur as a repetitive unit in certain *P. sojae* strains, including the isolate that was used for whole genome sequencing (P6497), thus accounting for the poor assembly of this region. Overall, our results can be integrated into a model whereby *Avr*1a copy number polymorphisms lead to transcriptional and translational differences that either activate or evade effector-triggered immunity on *Rps*1a soybean plants.

477. Identification of *Phytophthora sojae Avr3a* by Expression Profiling. Jennifer Tedman-Jones, Jonathan Eckert, and Mark Gijzen. Agriculture and Agri-food Canada, Southern Crop Protection and Food Research Centre, London, Ontario, Canada

Past studies have shown that some *Phytophthora sojae* isolates have lost expression of a particular avirulence (*Avr*) gene to evade recognition by soybean cultivars carrying the corresponding resistance (*R*) gene. To identify genes whose expression is associated with a particular avirulence trait, two F_2 bulks were generated from an existing *P. sojae* population (Race2: *Avr3a/Avr5/Avr3c* crossed with Race7: *avr3a/avr5/avr3c*) based on the virulence phenotype of individual progeny on cultivars carrying *Rps3a*, *Rps5* or *Rps3c*. The expression profiles of 15,800 transcripts were measured from total RNA, from infected soybean seedlings, for the two bulks and the two parents, using Affymetrix gene chips. The microarray data was mined to identify avirulence-associated transcripts. Transcripts that showed an association were further analyzed by RT- PCR of total RNA from individual F_2 progeny. RT-PCR analysis revealed one transcript that showed 100% association to avirulence, conditioned by *Avr3a* and *Avr5*, among the F_2 progeny and parents. This transcript encodes a small secreted peptide containing RXLR-dEER motif typical of oomycete avirulence genes. The corresponding gene is located in a region of the genome with a high density of genes encoding secreted peptides with the RXLR-dEER motif. Co-bombardment of the avirulence gene candidate with the promoter:reporter gene construct, 35S:beta-glucuronidase (GUS), results in a marked reduction in GUS activity in soybean plants carrying *Rps3a*, but not those carrying *Rps5*. This data shows the protein product of this candidate is specifically recognized by RPS3a in the soybean host, and therefore most likely encodes the *P. sojae* avirulence gene *Avr3a*.

478. Identification of secreted proteins from appressoria of *Colletotrichum higginsianum* by analysis of expressed sequence tags. Jochen Kleemann, Hiroyuki Takahara, Kurt Stueber, and Richard O'Connell. Max Planck Institute for Plant Breeding Research, Koeln, Germany. oconnel@mpiz-koeln.mpg.de

The hemibiotrophic ascomycete *Colletotrichum higginsianum* causes anthracnose disease on brassica crops and the model plant *Arabidopsis*. Melanized appressoria initially pierce the host cuticle and cell wall to form specialised biotrophic hyphae that develop inside living epidermal cells. The aim of this study was to identify proteins secreted by appressoria, which may function to overcome host defense responses and reprogram host cells for biotrophy. A random- primed cDNA library was prepared from mature appressoria formed *in vitro* and expressed sequence tags (ESTs) derived from sequencing 980 clones were assembled into 520 unique sequences. These were then screened for putative open reading frames containing signal peptides using the SignalP prediction program. Although based on a relatively small dataset, including some N-terminally incomplete sequences, this analysis identified 61 proteins with predicted signal peptides that are likely to be cotranslationally imported into the endoplasmic reticulum. Sequence similarity searches with BLASTP revealed that 37 of these had significant homology to proteins of known function, including some soluble secreted proteins and integral membrane proteins that have been implicated in fungal pathogenicity. A further 24 putative secreted

proteins were either homologous to fungal hypothetical proteins or lacked any database matches and are candidates for being novel effectors. The temporal expression of selected candidates during fungal development *in vitro* and *in planta* was examined using RT-PCR.

479. Translating and Coping with Stress: eIF2*α* kinases and the Cross-Pathway Control System of Aspergillus fumigatus.
Christoph Sasse[1], Elaine M. Bignell[2], Stanley Kim[3], Gerhard H. Braus[1], Sven Krappmann[1]. [1]Georg-August University Goettingen, Grisebachstr. 8, D-37077 Goettingen, Germany; [2]Imperial College London, UK; 3 The Institute for Genomic Research, USA

Aspergilli represent unique pathogens with *Aspergillus fumigatus* being the predominant perpetrator. We are interested in nutritional requirements sustaining propagation and supporting virulence. Fungal amino acid (aa) biosynthesis is regulated on various levels; besides pathway-specific systems, one global regulatory network has evolved that acts on aa metabolism as a whole. In its very components, this *C*ross-*P*athway *C*ontrol is made up by an eIF2 kinase sensing aa deprivation and translating it into increased levels of the transcriptional activator protein CpcA, which in turn elevates transcription for the majority of amino acid biosynthetic genes. As deduced from profiling studies, the scope of the CpcA-directed transcriptome exceeds amino acid biosynthesis. To elucidate the role of the CPC signal transduction cascade, the gene of the sensor kinase CpcC was cloned. In contrast to *cpcAdelta* mutants, strains deleted for *cpcC* are not impaired in virulence, indicating that the basal expression level of CpcA is necessary and sufficient to support pathogenesis. Western blot analyses indicate that the *cpcC*-encoded kinase is not required exclusively to phosphorylate the eIF2 subunit. Upon inspection of the *A. fumigatus* genome, the presence of a related gene (*ifkB*, for initiation factor kinase) could be revealed. The *ifkB* gene was deleted in and corresponding mutant strains are currently evaluated.

480. Gene expression of dermatophyte *Trichophyton rubrum* during assimilation of keratin in mimetic infection.
Maranhão, FCA, Paião, FG, Martinez-Rossi, NM. Departamento de Genética, Faculdade de Medicina de Ribeirão Preto-USP Av Bandeirantes 3900, 14049-900 Ribeirão Preto-SP, Brazil E-mail: fcam@usp.br

***T. rubrum* is a cosmopolitan and antropophilic fungus, the most prevalent in superficial mycoses, growing preferentially in keratin substrates as skin and nail. This work evaluated the pH changes during *T. rubrum* growth in keratin (after 6, 12, 24, 48, 72h and 7 days) at initial pH 5.0. *T. rubrum* promoted a medium pH increase reaching 8.3 in keratin, after 72h. However, this effect was not observed when the fungus was cultivated in glucose as carbon source, where the pH was maintained in approximately 5.0. Also, we identified 576 *T. rubrum* transcripts differentially expressed by Subtractive Suppression Hybridization using mycelia cultivated in keratin (72h) as tester and in glucose as driver. Clusters were recovered with CAP3 sequence assembly program, yielding 40 contigs and 215 singlets. The ESTs sequences were analyzed against the protein databases in GenBank by BLASTx search. Putative proteins encoded by genes obtained under keratin condition showed similarity to several fungi proteins involved in basic metabolism, growth and virulence, i.e., transporters ABC-MDR, MFS and ATPase of copper, NIMA interactive protein, pol protein, virulence factors serine-protease subtilisin and metalloprotease, cytochrome p450, GlcN-6-phosphate deaminase and HSP30. The induction expression of 214 genes was confirmed by macro array dot blot, and the upregulation of *T. rubrum* genes of subtilisin, metalloprotease and pol protein were also validated by northern blot. Our results show the SSH efficacy to reveal genes upregulated in keratin and suggest a relationship between these proteins and *T. rubrum* pathogenicity. Financial Support: FAPESP, CAPES, CNPq, FAEPA**

481. Genomic discovery and evolutionary patterns of the effector proteins in *Phytophthora.*
Rays H.Y. Jiang[1,2,3], Francine Govers[1], Michael C. Zody[2], Chad Nusbaum[2], Sucheta Tripathy[3], and Brett M. Tyler[3]. [1]Laboratory of Phytopathology, PSG, Wageningen University, Wageningen, The Netherlands [2]Broad Institute of MIT and Harvard, Cambridge MA, USA [3]Virginia Bioinformatics Institute, Virginia Polytechnic and State University, Blacksburg VA, USA

Biotropic oomycetes and fungi secrete a variety of molecules, many of which presumably promote infection. Molecules with a role in virulence or pathogenicity are termed effectors. In *Phytophthora*, a genus that belongs to the oomycetes and comprises many notorious plant pathogens, several effectors have been identified that all possess a RXLR- dEER domain. Bioinformatic mining of the genome sequences of *Phytophthora sojae* and *Phytophthora ramorum* revealed around 350 RXLR-dEER effector-like genes in the each of the two species. These genes belong to the most rapidly evolving genes in the genome. They show a high level of sequence divergence between species as well as extensive lineage specific expansion. Moreover, the genomic regions where RXLR-dEER genes are located show significant enrichment of retrotransposons, and nearly always show features associated with genome rearrangements. Despite of their high sequence divergence, all the proteins in the large RXLR-dEER super-family seem to be related. A single ancestral molecule has most likely evolved into this super-family by rapid duplication and divergence. The rapid pace of evolution of the RXLR-dEER proteins underlines the potential role of these proteins in the interaction with host plants, whereas the conserved RXLR-dEER domain points to a functional feature shared by the members of this super- family.

482. The Galpha-subunit BCG1 – An activator of Ca2+/calcineurin-dependent signaling in *Botrytis cinerea*. Julia Schumacher [1], Muriel Viaud[2], and Bettina Tudzynski[1]. [1]Institut für Botanik, Westf. Wilhelms-Universität Münster, Schlossgarten 3, D-48149 Münster, Germany [2]Unité de Phytopathologie et Méthodologies de la Détection, INRA, Route de Saint-Cyr, F-78086 Versailles, France

The alpha-subunit BCG1 of a heterotrimeric G-protein plays an important role during the infection of host plants by the gray mold fungus *Botrytis cinerea*: BCG1 triggers the transition from primary infection to secondary invasive growth, is essential for the production of the phytotoxic secondary metabolite botrydial and for the secretion of proteases. Based on the fact that the expression of several BCG1 target genes is also dependent on the Ca2+/calmodulin-dependent calcineurin-phosphatase, we suggest that BCG1 is an upstream regulator of calcineurin, in particular in regulation of secondary metabolism. In order to identify the transcription factor downstream of BCG1 and calcineurin, we cloned the *B. cinerea* homologue of CRZ1 (Calcineurin-Responsive Zinc finger), the mediator of calcineurin function in yeast. The analysis of gene expression in the corresponding deletion mutants confirmed our suggestion that this transcription factor acts downstream of calcineurin. Furthermore, we propose a model in which the interconnection between BCG1 and calcineurin is mediated by BcPLC1, a phosphatidylinositol-specific phospholipase C. Mutants with educed transcript levels of *bcplc1* showed the same alteration in gene expression as *bcg1*- and *bccrz*-mutants.

483. The cAMP-signaling cascade is required for full pathogenicity in *Botrytis cinerea.* Julia Schumacher, Christina Huesmann, and Bettina Tudzynski. Institut für Botanik, Westf. Wilhelms-Universität Münster, Schlossgarten 3, D-48149 Münster, Germany

It was shown that the highly conserved cAMP signaling pathway plays a crucial role during pathogenesis in the plant infecting fungus *Botrytis cinerea*. The upstream components of this cascade were analysed in detail: There are three different alpha-subunits of heterotrimeric G-proteins and two of them, BCG1 and BCG3, are activators of the adenylate cyclase BAC, the producer of cAMP. The characterization of the corresponding deletion mutants showed that this pathway regulates morphogenesis (sugar concentration-dependent colony morphology), germination of conidiospores (reduced sugar dependent germination) and virulence on bean plants (retarded infection process). To study the cAMP-pathway in more detail, we focused on the cAMP-dependent protein kinase (PKA), the main effector of the adenylate cyclase and cAMP. In the *B. cinerea* genome, two catalytic subunit encoding genes (*pka1* and *pka2*) and one gene encoding the regulatory subunit (*pkaR*) were identified. While the *pka1*- mutants show a general growth defect and reduced virulence similar to *bac*-mutants, *pka2*-mutants have no obvious phenotype. In contrast, mutants with a "dominant-active" PKA, resulting from the knock out of the regulatory subunit, revealed a slow growth rate and repressed sporulation. Interestingly, some features of the *bac*-mutant differ from those of the *pka1*-mutant suggesting crosstalks to other signaling pathways.

484. Comparisons of the morphology and pathogenicity of *Fusarium graminearum* strains PH1 and Gz3639. S. Bec[1], D. Van Sanford[2], L. Vaillancourt[1]. [1]Department of Plant Pathology, [2] Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY

***Fusarium graminearum* is a major causal agent of Fusarium Head Blight (FHB) of small grains. The genome sequences of two *F. graminearum* strains isolated from wheat heads, PH1 (NRRL 31084) and Gz3639, have been assembled and released. In spite of the high level of overall genome sequence homology, the two strains differ significantly in colony morphology, growth rate, production of asexual spores, fertility, and mycotoxin profiles. Apart from deoxynivalenol (DON), which is produced by both strains, PH1 also produces 3-ADON while Gz3639 belongs to the 15-ADON chemotype. We used the point inoculation method of infecting wheat heads at anthesis to compare pathogenicity and aggressiveness of these two strains at different inoculum concentrations on three wheat varieties. The wheat varieties differ in the degree and type of resistance they express against FHB. Both of the Fusarium graminearum strains produced similar disease symptoms at high (5×10^6 and 5×10^5 spore/ml) inoculum concentrations. However, significant differences in disease severity and the rate of symptom development became evident at lower (5×10^3 spore/ml) spore concentrations, with PH1 being more aggressive than Gz3639. It has recently been reported that 3-ADON chemotype strains (like PH1) are more aggressive on wheat. Our goal is to analyze the progeny of a cross between PH1 and Gz3639 in order to test the hypothesis that the higher level of aggressiveness displayed by PH1 is due to production of 3-ADON.**

485. Discovering differentially regulated genes in the lichen symbiosis. Suzanne Joneson[1], and Francois Lutzoni[1]. [1]Duke University, Biology Department, Box 90338, Durham NC 27708, USA.

Lichens are the symbiotic association of fungi (mycobionts) with green algae and/or cyanobacteria (photobionts). Although one fifth of all known fungi form obligatory lichens with photobionts, we know nothing of the genetic or molecular mechanisms underlying this life-style. Here we present the first investigations into differentially expressed

genes in early lichen development including pre-contact, and initial contact of the symbionts. Using the fungus *Cladonia grayi* and the green alga *Asterochloris* sp., we have sequenced over 2000 fungal and algal clones upregulated in response to resynthesized symbiosis. We used BLAST and FASTA algorithmic searches of databases containing nucleotides, proteins, and conserved protein domains to characterize our sequences. The data obtained provides insights into similarities between *C. grayi*'s response to it's photosynthetic host, and that of phytopathogenic fungi to their plant hosts. This dataset represents the first global survey of fungal and algal gene sequences involved in lichen symbiosis, and a summary of these genes and their putative functions will be presented. The results of this study will allow us to identify candidate genes of early lichen development for future research.

486. Different secreted lipases are virulence factors of *F. graminearum*. Nguyen Nam L., M. Fehrmann, S. Salomon, W. Schäfer. University of Hamburg, Biocenter Klein Flottbek, Ohnhorststr. 18, Hamburg, Germany

***Fusarium graminearum* (teleomorph *Gibberella zeae*) is the major cause of Fusarium head blight on wheat, barley and other small grain cereals and of ear rot on maize. The outcome of infection varies between total destruction of the host and minor damage. Virulence of *F. graminearum* is the consequence of a collection of additively acting virulence factors. Only the trichothecene mycotoxins were known to be secreted virulence factors. We identified recently a secreted fungal lipase as a new virulence factor (Voigt et al. 2005), in addition to the already known secreted trichothecenes. Knowledge about the role of all virulence factors in promoting virulence and their distribution in different isolates will contribute to the understanding of the cereal – *Fusarium* interaction and offer new approaches to resistance breeding. We identified 17 putatively secreted lipase genes in the genome of *F. graminearum*. We cloned and characterized 5 of these genes. Three of them act as virulence factors, whereas a forth secreted lipase is not involved in fungal virulence. A fifth lipase seems to uphold vital functions in the fungal cell, as we were unable to disrupt the gene. We show the infection behaviour of the lipase-mutants on wheat and a transcriptional analysis of the lipase genes in culture and during wheat head infection. Voigt, C.A., Schäfer, W., and Salomon, S.: A secreted lipase of *F. graminearum* is a novel virulence factor during infection of cereals. Plant J. 42, 364–375 (2005).**

487. Distinct amino acids of *Phytophthora infestans* AVR3a condition activation of R3a hypersensitivity and suppression of cell death. Jorunn I. B. Bos, Angela Chaparro-Garcia, Lina M. Quesada, Sophien Kamoun. Dept. of Plant Pathology, Ohio State University-OARDC, Wooster, OH, USA

The AVR3a effector of *Phytophthora infestans* is a member of the RXLR family of cytoplasmic effectors that exhibits dual effector functions. AVR3a induces hypersensitivity mediated by the resistance protein R3a and suppresses cell death induced by *P. infestans* INF1 elicitin. Two polymorphic forms of AVR3a that differ in two amino acids in the mature region occur in *P. infestans*. While AVR3aKI can mediate both R3a activation and cell death suppression, no effector function has been identified for AVR3aEM. To gain insights in the molecular basis of AVR3a activities, we performed structure-function analyses of both forms of AVR3a. We used near saturation high-throughput random mutagenesis screens based on functional expression in *Nicotiana benthamiana* to identify *Avr3a* mutants with altered activation of R3a hypersensitivity. Out of more than 6500 AVR3aEM mutants tested, we identified about 150 gain-of-function mutants that induced R3a hypersensitivity. About 10 residues, mostly charged amino acids, were frequently substituted in these mutants. These AVR3aEM gain of function mutants did not always gained the ability to suppress cell death suggesting that distinct residues condition R3a hypersensitivity and cell death suppression. A similar loss of function screen of 4500 AVR3aKI mutants resulted in smaller number of mutants with altered activity. These results point to a model that involves the interaction of AVR3a with a host protein and is not consistent with the recognition of AVR3a through an enzymatic activity.

488. Roles of MFS and ABC transporters in cercosporin resistance and biosynthesis in the fungus *Cercospora nicotianae*. Alongkorn Amnuaykanjanasin and Margaret E. Daub. Department of Plant Biology, North Carolina State University, Raleigh, NC, U.S.A.

Cercosporin, a photoactivated toxin produced by *Cercospora* spp., is highly toxic to a wide range of organisms due to its production of reactive oxygen species. The producing fungus has self-resistance to its own toxin. However, a *C. nicotianae* mutant disrupted in the transcriptional regulator gene *CRG1* is sensitive to cercosporin and also produces approximately a half of the toxin produced by wild type (WT). This mutant (crg1) also showed increased green fluorescence in the mycelium, indicative of accumulation of the endogenous, reduced form of cercosporin. We hypothesized that efflux pump transporters may not be functioning properly in crg1. A subtractive library was obtained of genes differentially expressed between crg1 and WT. From this library we focused on genes encoding major facilitator superfamily (MFS) and ATP-binding cassette (ABC) transporters to determine their possible roles in self-resistance and production of cercosporin. We cloned and sequenced two MFS transporter genes (*CnCFP* and *MFS2*), the former having homology to cercosporin facilitator protein (CFP) reported to be the cercosporin efflux pump in *C. kikuchii*. Overexpression of these two MFS genes in the crg1 mutant did not increase either cercosporin resistance or production. We are currently disrupting three

ABC transporter genes (*ABC1*, *ABC2*, and *ABC3*) in the WT. We also determined that the crg1 mutant produces cercosporin in complete medium (CM) liquid culture, but none in potato dextrose broth (PDB). Quantitative RT-PCR data indicated that whereas all of the transporter genes studied were down-regulated in crg1 grown in PDB, the three ABC transporters were up-regulated in CM.

489. Elicitation of systemic induced resistance by *Trichoderma virens.* Charles Kenerley, Dept. Plant Pathology & Microbiology, Texas A&M University, College Station, Texas

We have isolated a small hydrophobin-like protein (SM1) and an 18 amino acid peptaibol (TVB group) from *Trichoderma virens* and have shown that they are elicitors of systemic disease resistance in several plants. The native, purified SM1 elicits plant defense responses and systemic resistance against a foliar pathogen, *Colletotrichum*. The protective activity of SM1 is associated with the accumulation of reactive oxygen species and phenolic compounds, and increased levels of transcription of the defense genes regulated by salicylic acid (SA) and jasmonic acid (JA)/ethylene (ET), as well as genes involved in the biosynthesis of sesquiterpenoid phytoalexins. Similar as in dicots, colonization of maize roots by *T. virens* induces systemic resistance (ISR-like) in leaves challenged with *C. graminicola*. This protection is associated with induction of JA and green leaf volatiles biosynthetic genes. We have demonstrated that SM1 is required for activation of ISR in maize as plants grown with *sm1* deletion strains exhibit the same levels of systemic protection as plants without *T. virens*. Conversely, plants grown with *sm1* over-expressing strains displayed an increased protection compared to the wild type strain. The application of two synthetic peptaibol isoforms to cucumber seedlings induce systemic protection against the bacterial pathogen *Pseudomonas syringae pv. lachrymans*, stimulate production of antimicrobial compounds in cotyledons, and upregulate the expression of defense-related genes. Additionally, cucumber plants co-cultivated with fungal strains disrupted in *tex1* show reduced ability to produce phenolic compounds with inhibitory activity towards the bacterial pathogen as compared to those grown with the WT.

490. RNA Silencing Functions as an Antiviral Defense Mechanism in the Chestnut Blight Fungus *Cryphonectria parasitica.* Xuemin Zhang, Gert Segers, Qihong Sun and Donald Nuss*. Center for Biosystems Research University of Maryland Biotechnology Institute Shady Grove Campus 9600 Gudelsky Dr. Rockville, MD 20850 *E-mail: nuss@umbi.umd.edu

RNA silencing pathways are conserved in animals, plants and fungi. Although RNA silencing has been shown to play a crucial role as an antiviral defense mechanism in plants and to influence virus replication in animal cells, the interaction between mycoviruses and RNA silencing in fungi is not well understood. We recently reported that the p29 protein encoded by the virulence-attenuating hypovirus CHV1-EP713 can suppress RNA silencing in the natural host, the chestnut blight fungus *Cryphonectria parasitica*. (Segers et al., 2006, Eukaryotic Cell, 5:896-904). We now report the cloning and characterization of hypovirus-derived small RNAs (vsRNAs) from CHV1-EP713 infected *C. parasitica*. The vsRNAs were distributed along the viral genome and were derived from both the positive and negative viral RNA strands with a ratio of 3:2. These results suggest that hypovirus vsRNAs are derived from both structured regions of the positive strand and from viral double-stranded RNA, which contrasts with reports that vsRNAs are derived primarily from the positive strand RNA of several plant viruses. We also report the cloning and disruption of an Argonaut and two Dicer genes from *C. parasitica*. While the disruption mutants did not exhibit any obvious phenotypic changes relative to the wild-type strain, CHV1-EP713 infection resulted a dramatic debilitation of the DCL-2 disruption mutant. The identification of hypovirus derived vsRNAs and demonstration of enhanced virus-mediated symptom expression upon disruption of the *C. parasitica* RNA silencing pathway provides the first experimental evidence that RNA silencing serves as an antiviral defense mechanism in fungi.

491. Effect of ToxA treatment on wheat leaf gene expression. Iovanna Pandelova[1], Todd Mockler [1], Lynda Ciuffetti[1]. [1]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR

The fungal wheat pathogen *Pyrenophora tritici-repentis* (PTR) produces several proteinaceous host-selective toxins (HSTs). The HSTs of *P. tritici-repentis* represent compounds that evoke a susceptibility response in the host in a single-gene dependent manner. Understanding the nature of events triggered by HSTs in the host will provide valuable insight into the nature of host disease susceptibility. ToxA is one of the proteinaceous HSTs produced by PTR that has been well characterized. ToxA treatment of a sensitive cultivar produces necrotic symptoms similar to those produced by the pathogen. Previous studies in our laboratory suggest that ToxA is imported by an endocytotic process and then localized to chloroplasts. *In vitro* studies suggest that ToxA interacts with the chloroplast protein ToxABPI thought to play a role in thylakoid formation or regulation of photosystem II. The downstream events of this interaction and other processes involved in the susceptible response in the host are likely to require differential gene expression. In the present study, we provide microarray data analysis for genes expressed in ToxA- and mock-treated leaves of a ToxA-sensitive cultivar over time. We used Wheat Genome Arrays from Affymetrix. Normalization and filtering of the data was done with Bioconductor software packages and relational database queries. Differentially expressed genes were identified between each treatment at each time point with three different statistical methods.

492. Internalization of Ptr ToxA is facilitated by the RGD-containing, solvent exposed loop. Viola Manning[1], Sara Hamilton[1], P. Andrew Karplus[2], and Lynda Ciuffetti[1]. [1]Department of Botany and Plant Pathology, [2]Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR

Pyrenophora tritici-repentis **produces host-selective toxins (HSTs) that induce the symptoms of tan spot of wheat. Expression and secretion of Ptr ToxA (ToxA), the first proteinaceous HST to be isolated and characterized, results in necrotic lesions on ToxA-sensitive leaves. Necrosis also occurs when ToxA-sensitive leaves are treated with purified toxin. Internalization of ToxA into sensitive, but not insensitive mesophyll cells suggests that internalization of toxin is required for activity. The cloning of the ToxA gene and its subsequent expression in *E. coli* results in an active HST and provides a method to investigate the structure/function relationship of ToxA. Crystal structure analysis indicates that an RGD-containing loop in ToxA is available for protein-protein interaction and heterologously expressed proteins with mutations in this loop are less active. We sought to determine if the reduced activity of these mutant proteins was due to decreased internalization. By monitoring toxin internalization with a protease protection assay we were able to determine that the decrease in toxic activity was due to a decrease in internalization and the amount of internalized protein correlates with the intensity of symptoms induced.**

493. Medical Mycology in the graduate and undergraduate curriculum. Thomas Volk University of Wisconsin-La Crosse, Biology

Although fungi are increasing in importance as more patients become immunocompromised and acquire fungal infections, Medical Mycology is not a common course in undergraduate or graduate programs, with fewer than 40 full-fledged Medical Mycology courses currently taught in North America. However, Medical Mycology can be an interesting and popular course if taught and "marketed" to students in certain ways. This presentation is the story of a successful Medical Mycology course at the University of Wisconsin-La Crosse (about 9000 students) where enrollment one semester peaked at 38 students. I started with an enrollment of 13 students in Spring 1997; there have been about 260 students in the past 11 years. Through word of mouth and "advertising," enrollments have steadily increased, especially among pre-med students, when they realize they will receive very little training in medical mycology in medical school. My course is taught mostly from the fungus' point of view, although we do discuss the symptoms and cures for each disease, as well as mycetismus and mycotoxicosis. The laboratory is the crux of the course; each student makes a permanent reference slide collection of approximately 45 species of pathogenic and contaminant fungi, mostly deuteromycetes, using PVLG mounts of slide cultures or tape mounts. Students learn the methodology to identify just about any unknown deuteromycete, a very important and sought-after job skill. We have a poster session where each student presents a poster on a self-chosen topic, similar to my fungal web pages at TomVolkFungi.net . Most importantly, it's a fun course to teach, and students enjoy it a lot.

494. *Fusarium verticillioides GAS1*, a gene encoding glycolipid anchored surface protein, is involved in growth and conidiation. Uma Shankar Sagaram, Brian D. Shaw, and Won-Bo Shim. Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX77843-2132, USA

Fusarium verticillioides **is an important pathogen of maize that causes ear rot and produces mycotoxins fumonisins. To date, our knowledge about the pathogenicity and the regulation of fumonisin biosynthesis in *F. verticillioides* is limited. Here, we present the molecular characterization of *GAS1*, which encodes a putative 540-amino acid protein. Gas1 protein belongs to a glycolipid anchored surface protein (GASP) family. *F. verticillioides GAS1* was identified as an expressed sequence tag (EST) up-regulated in the acidic culture condition. *GAS1* was shown to be involved in fungal cell wall biogenesis and virulence in *F. oxysporum* and *A. fumigatus*. *GAS1* deletion mutants (*gas1-h* and *gas1-g*) grew restricted but fluffier when compared to the wild type on solid media. However, no defect with regard to mycelial mass production and filamentous growth was observed when the strains were grown in liquid media. The deletion strains produced significantly fewer conidia than its wild type progenitor. Microscopic studies as visualized by concanavalin-A staining indicated that the deletion of *GAS1* altered the cell wall carbohydrate composition/deposition process. Interestingly, the deletion of *GAS1* did not affect pathogenicity of *F. verticillioides* when tested on maize seedlings and stalks. Complementation of *gas1-h* with *GAS1* restored growth, conidiation and cell wall abnormality phenotypes. Our results suggest that *GAS1* is associated with growth, development and conidiation in *F. verticillioides* but not pathogenicity. The role of *GAS1* in fumonisin biosynthesis will also be discussed the presentation.**

495. Loss of pathogenicity converts a pathogenic *Fusarium oxysporum* isolate to a potential biocontrol agent in *Arabidopsis thaliana.* Hye-Seon Kim[1], Kirk Czymmek[2] and Seogchan Kang[1]. [1]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802,USA. [2]Department of Biological Sciences, University of Delaware, Newark, DE 19716,USA.

The soil-borne fungal pathogen *Fusarium oxysporum* can cause vascular wilt and root rot diseases in many crop species. To facilitate the investigation of the molecular and cellular mechanisms underpinning its pathogenesis and host responses, a new model system was developed using *Arabidopsis thaliana* as a host. *In vivo* observations of root colonization were facilitated by transformation of *F. oxysporum* for constitutive cytoplasmic expression of fluorescent protein reporters. In combination with these reporters, we investigated the role of the cAMP-dependent protein kinase A (*CPKA*) in pathogenicity. Targeted knock-out mutagenesis of *CPKA* caused the loss of pathogenicity in *Arabidopsis* and the failure to colonize the vascular system and also led to reduced vegetative growth and conidiation in culture. It was observed that the coinoculation of the wild-type strain with a *cpka* mutant significantly reduced the disease severity and the colonization of the vascular system by the wild-type strain, indicating that the *cpka* mutant protects *Arabidopsis* plants from infection. Molecular cytological tools and gene expression analyses are currently being used to study the role of *CPKA* during root pathogenesis, how the loss of this gene function protects plants from a virulent strain, and whether the loss of function of other pathogencity genes convert pathogenic isolates to potential biocontrol agents.

496. Habitat-defining genes and synteny of conditionally dispensable (CD) chromosomes in *Nectria haematococca*. Marianela Rodriguez-Carres, Dai Tsuchiya, Jose Luis Goicoechea, Rod Wing, and Hans D. VanEtten. Department of Plant Science, University of Arizona, Tucson, AZ 85721.

Many studies of extra chromosomes in plant and animals have described them as detrimental or parasitic, and containing non-coding or non-expressed DNA. However, in fungi many of these chromosomes are required for pathogenicity but not for axenic growth, and as such they have been called conditionally dispensable (CD). The wide range of habitat of the plant pathogenic fungus *Nectria haematococca* is partially attributed to the presence of CD chromosomes. One of these CD chromosomes, the 1.6Mb *PDA1*- CD chromosome, contains a cluster of genes for pea pathogenicity and rhizosphere colonization. In the current study a physical map of the *PDA1*-CD chromosome was constructed in order to evaluate synteny between this CD chromosome and another CD chromosome, the *MAK1*-CD. The *MAK1*-CD chromosome has a similar size to the *PDA1*-CD chromosome, and contains a gene (*MAK1*) for pathogenicity on chickpea. Results show that about 463kb (>89% sequence identity) are shared by both CD chromosomes but not found anywhere else in the genome. In addition, each chromosome has ~1 Mb of unique DNA that is unshared. Anchoring of the *PDA1*-CD map to the genome of *N. haematococca* allowed the identification of additional putative habitat-defining genes present on both CD chromosomes. Southern hybridizations of pulsed-field gels and sequence analyses of the syntenic regions reveals features reminiscent of recombination/translocation events, and a mosaic composition of the genes in these CD chromosomes. Taken together, these results indicate that: CD chromosomes are a reservoir of unique DNA, advocates for a common ancestor, and suggests possible mechanisms that lead to the formation of CD chromosomes.

497. Comparative analysis of the *MAT* locus to understand speciation and sexual cycle transitions: *Cryptococcus neoformans* vs. *Filobasidiella depauperata*. Marianela Rodriguez-Carres, Keisha Findley and Joseph Heitman. Duke University Medical Center, Department of Molecular Genetics and Microbiology, Durham, NC 27707.

Cryptococcus neoformans* and *Cryptococcus gattii* are dimorphic basidiomycetes that cause respiratory and neurological disease in animals and humans, following spore inhalation from the environment. Sexual reproduction in these fungi is controlled by a bipolar system in which a single mating type (*MAT*) locus specifies compatibility. The *MAT* loci in *C. neoformans* and *C. gattii*, which are thought to have evolved from a common ancestral locus, share several features reminiscent of sex chromosomes in multicellular eukaryotes. They are unusually large (>100 kb), do not recombine during meiosis, and contain many genes clustered together, most of which function during mating whereas others may not. The alpha mating type (*MAT* alpha) is overwhelmingly prevalent, both in nature and clinical isolates, and has been associated with increased virulence in certain genetic backgrounds. The abundance of *MAT* alpha isolates could be partially attributed to their ability to undergo monokaryotic fruiting which is a variant of the sexual cycle that involves same-sex mating and results in the production of basidiospores. Consequently it has been proposed that the evolution of the *MAT* locus could be closely linked to the pathogenic potential of *C. neoformans* and *C. gattii*. To gain insight into the evolution of the *MAT* locus and monokaryotic fruiting, we are cloning the *MAT* locus from the closest known relative *Filobasidiella depauperata*. This fungus is homothallic, exclusively filamentous and found in associations with insects. Preliminary results suggest that several genes linked to the *MAT* locus in *Cryptococcus* are unlinked in *F. depauperata*. This is intriguing, as it has been proposed that the ancestral *MAT* locus of *C. neoformans* might have evolved through a chromosomal translocation that clustered all of the mating genes in one region. Furthermore, morphological characterization of *F. depauperata* shows that clamp connections are absent, and monokaryotic basidiospores and hyphae are present, features that resemble monokaryotic fruiting of *C. neoformans.

498. Recovery and functional analysis of a putative pathogenicity gene in the Dutch Elm Disease fungus *Ophiostoma novo-ulmi*. Karine Plourde, Louis Bernier. Laval University, Québec, Canada.

The ascomycete fungus *Ophiostoma novo-ulmi* is the main causal agent of Dutch Elm Disease. We used insertional mutagenesis to tag genes that may contribute to parasitic fitness in this fungus. We recovered several transformants with a significantly reduced pathogenicity phenotype compared to the aggressive wild-type reference strain H327. Analysis of a genomic library for one of these mutants allowed us to identify 4 genes next to the mutation site, including a locus with high homology with the *Aspergillus fumigatus* ubiquitin-protein ligase E3. To evaluate if the mutation modified the expression of these genes during infection of the host plant by *O. novo-ulmi*, the mutant and its wild-type progenitor were inoculated to elm saplings under controlled conditions. We used quantitative RT-PCR to estimate fungal biomass and gene expression in elm leaves collected at 7, 14, 21 and 42 days after inoculation. This allowed us to verify if differences in the expression level between strains were associated with differences in their ability to colonize elms. In addition, wood xylem samples collected 42 days after inoculation were used for histopathological analyses. Preliminary observations showed a stronger defense response (suberisation, phenol production) in plants inoculated with the wild-type strain than with the mutant. Overall, our results suggest that activity of the ubiquitin-protein ligase E3 locus, or one of its immediate neighbors, is important for the virulence of *O. novo-ulmi* towards elms.

499. Functional Analysis of *MPEX7*, the putative peroxisome-targeting signal type 2 (PTS2) receptor gene of *Magnaporthe oryzae*. Jaeduk Goh, Hyojeong Kim and Yong-Hwan Lee. Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea.

Peroxisomes are single-membrane-bound organelles that function in multiple metabolic pathways and, as recently demonstrated, influence appressorium-mediated penetration of pathogenic fungi to host plants. A putative peroxisomal targeting signal type-2 (PTS2) receptor gene, *MPEX7*, of the rice blast fungus *Magnaporthe oryzae* was identified through our *Agrobacterium tumefaciens* -mediated transformation project. *mpex7* mutants generated by a T-DNA insertion or a targeted gene replacement showed significant reductions in agar-invasive hyphal growth, conidiophore differentiation and conidiation, while displaying normal rates in conidial germination and hyphal extension. They formed normal-appearing appressoria, however, the appressoria of the mpex7 mutants failed to cause disease in plant cells, due to inability to develop penetration hyphae. *mpex7* mutants caused lesions only if inoculated through wound sites. Moreover, the appressoria proved to be defective in turgor pressure generation and delayed in lipid degradation during appressorial maturation. Thus, our results demonstrate that *MPEX7* is required for the formation of functional appressorium and pathogenicity of *M. oryzae*.

500. The effectors Avr2 and EPIC2B secreted by two unrelated pathogens target the tomato defense protease Rcr3. Jing Song, Joe Win, Miaoying Tian, Hsin-Yen Liu and Sophien Kamoun Department of Plant Pathology, The Ohio State University-OARDC, Wooster, OH 44691, USA

Current models of plant-pathogen interactions stipulate that pathogens secrete effector proteins that disable plant defense components known as virulence targets. Occasionally, the perturbations caused by these effectors trigger innate immunity via plant disease resistance proteins as described by the "guard model". This model is nicely illustrated by the interaction between the fungus *Cladosporium fulvum*and tomato. *C. fulvum* secretes a protease inhibitor Avr2 that targets the tomato cysteine protease Rcr3. In plants that carry the resistance protein Cf2, Rcr3 is required for resistance to *C. fulvum* strains expressing Avr2, thus fulfilling one of the predictions of the guard model. The model has two other predictions that have not been tested. First, Rcr3 could be disabled by different pathogens. Second, Rcr3 may directly contribute to basal defense. In this study we tested these two predictions using a different pathogen of tomato, the oomycete *Phytophthora infestans*. This pathogen secretes an array of protease inhibitors, one of which, EPIC2B, was shown to inhibit tomato cysteine proteases. Here, we showed that, similar to Avr2, EPIC2B binds and inhibits Rcr3 using co-immunoprecipitation and activity profiling assays. We also found that the *rcr3-3* mutant of tomato that carries a premature stop codon in *Rcr3* exhibits enhanced susceptibility to *P. infestans* suggesting a role for Rcr3 in basal defense. In conclusion, our findings fulfill the predictions of the guard model and suggest that the effectors Avr2 and EPIC2B secreted by two unrelated pathogens of tomato target the same defense protease Rcr3.

501. The initiation of map-based cloning of an avirulence gene from *Pyrenophora teres.* Zhaohui Liu[1], Justin D. Faris [2], Michael C. Edwards[2], Timothy L. Friesen[2]. [1]Department of Plant Pathology, North Dakota State University, Fargo ND, 58105 [2]USDA-ARS Cereal Crops Research Unit, Northern Crop Science Laboratory, 1307 18th Street North, Fargo ND, 58105.

The fungus *Pyrenophora teres*, causal agent of barley net blotch, is an economically important pathogen in most barley-growing areas. In a previous study, we identified and mapped a fungal gene associated with avirulence on Tifang barley that was already designated *AvrHar*. This study used a *P. teres* mapping population consisting of parental isolates 15A (avirulent on Tifang) and 0-1 (virulent on Tifang). In the present study, an attempt is being made to clone *AvrHar* using a map-based approach. Initially, the fungal population used in the mapping of *AvrHar* was enlarged from 78 progeny to a total of 278 progeny to create a map with increased resolution. A single AFLP marker closely linked to the avirulence

gene was identified and TAIL-PCR was used to extend the fragment from 250 bp to 1.7kb for use as a probe. A 20X BAC library was constructed from parental isolate 0-1 and a 3X fosmid library was constructed from parental isolate 15A. The AFLP probe allowed the identification of 14 positive clones from the 0-1 BAC library. A single BAC clone was sequenced and shown to contain a large portion of Gypsy-like LTR- retrotransposon repeats at both ends. A 60kb region in the middle of the BAC contained several predicted genes. A PCR marker based on one of the genes located approximately 50 kb from the original AFLP was found to co-segregate with the AFLP marker, indicating that *AvrHar* has not been reached. An additional four BAC clones have been identified using the new marker. Twelve fosmid clones spanning approximately 90 kb outside the BAC clone sequence have been characterized. Recombination in this region is lower than expected and several walking steps may be necessary. Additional steps are being taken to span *AvrHar*.

502. Putative G protein-coupled receptors GPRC and GPRD are involved in development and morphogenesis in *Aspergillus fumigatus.* Alexander Gehrke[1], Thorsten Heinekamp[1] and Axel A. Brakhage[1,2]. [1]Leibniz Institute for Natural Product Research and Infection Biology - Department of Molecular and Applied Microbiology – Hans-Knoell-Institute, [2]Friedrich-Schiller-University, Jena; eMail:alexander.gehrke@hki-jena.de

The opportunistic human-pathogen *Aspergillus fumigatus* was subject to recent studies on cAMP signal transduction with regard to morphogenesis and virulence. To date, one of the most important questions is still unanswered: what are the external signals and the corresponding proteins sensing those ligands or stimuli which enable the fungus to grow in a wide variety of different ecological niches? In a first approach, two genes encoding putative G protein-coupled receptors *gprC* and *gprD*, designated as carbon source-sensing receptors, were deleted in *A. fumigatus*. The physiological characterisation of the mutants revealed altered growth on solid media. However, various growth conditions, which included the use of different carbon- and nitrogen-sources, did not restore the defect of the mutants. Virulence of the mutant strains, as tested in a low-dose murine infection model, was attenuated. The function of the putative GPCRs was further investigated by analysing fluorescent protein-fusions *in vivo* by confocal microscopy.

503. A+T-rich isochores as niches for avirulence and pathogenicity genes in the genome of *Leptosphaeria maculans.* I. Fudal[1], B. Profotova[1], S. Ross[1], L. Gout[1], F. Blaise[1], M.L. Kuhn[1], M. Eckert[1], L. Cattolico[2], S. Bernard-Samain[2], M.-H. Balesdent[1], T. Rouxel[1]. [1]INRA-PMDV, Route de St Cyr, 78016 Versailles, France. [2]Genoscope, 2 rue Crémieux, 91057 Evry, France.

***Leptosphaeria maculans* develops very specific "gene-for-gene" interactions with its host plant, *Brassica napus*, where fungal avirulence (*AvrLm*) genes are the counterpart of plant resistance (*Rlm*) genes. A 1.1 Mb region encompassing avirulence genes *AvrLm1* and AvrLm6 was sequenced and shown to display a particular organization, with five 20–70kb G+C-equilibrated, ORF-rich isochores intermingled with 3 large (170-450 kb) A+T-rich isochores mainly composed of degenerated and truncated retrotransposons. These A+T-rich regions contain only seven isolated genes (one every 90 kb on average), of which four are predicted to encode small-secreted proteins. These include AvrLm1 (205 aa, one cysteine), AvrLm6 (144 aa, 6 cysteines), and two single-copy genes encoding for two cysteine-rich proteins termed LmCys1 (233 aa, 8 cysteines) and LmCys2 (247 aa, 8 cysteines). Except for *LmCys1*, all these genes have a low G+C content, similar to that of their genomic environment. Contrasting with genes occurring in GC-equilibrated regions of the genome, *AvrLm1*, *AvrLm6*, *LmCys1* and *LmCys2* are specific of *L. maculans* 'brassicae', and absent from other species or sub-species of the *L. maculans*-*L. biglobosa* species complex, or from the closely related species, *Stagonospora nodorum*. These four genes are up-regulated upon plant infection, with *AvrLm1*, *AvrLm6* and *LmCys2* being expressed at a low level, but over-expressed up to 800-x in the first stages of plant infection. Functional analyses, including RNAi silencing or complementation, suggested a role for *LmCys1* and *LmCys2* in pathogenicity of *L. maculans* and confirmed that A+T-rich isochores are specific niches for genes involved in pathogenicity or avirulence. B. Profotova and M. Eckert were funded by Marie-Curie Fellowship HPMT-CT-2001-00395 'FUNGENE' I. Fudal and S. Ross were funded by EU contract QLK5-CT-2002-01813 'SECURE'**

504. The *Phytophthora infestans* avirulence gene *PiAvr*4 encodes an RxLR-DEER effector protein. Pieter M.J.A. van Poppel[1], Jun Guo[1,2], Peter J.I. van de Vondervoort[1], and Francine Govers[1]. [1] Laboratory of Phytopathology, Wageningen University, The Netherlands [2] IVF-CAAS Beijing and Northwest A & F University, Yangling Shaanxi, China

The oomycete pathogen *Phytophthora infestans* causes late blight, an important disease in potato world wide. *P. infestans* secretes numerous effector molecules, some of which are recognized by host plants carrying resistance (*R*) genes. These effectors then act as avirulence (*Avr*) factors and elicit a hypersensitive response (HR) that arrests growth of the pathogen. Through a combined approach of genetic mapping and transcriptional profiling (cDNA-AFLP) we isolated an *Avr* gene that shows a gene-for-gene interaction with *R4*. This gene, named *PiAvr*4, is highly expressed in germinated cysts and encodes a 287 amino acid protein with a putative signal peptide and an RxLR-DEER motif. The Avr4 protein belongs to a large family of *P. infestans* effector proteins that are highly divergent but share the RxLR-DEER motif. This motif is thought to play a role in delivery of effectors into the host cell. Transformation of *PiAvr*4 into *P. infestans* isolates virulent

on *R4* plants, resulted in complementation, i.e., the transformants elicited HR and thus became avirulent on plants carrying *R4*. *In planta* expression of *PiAvr*4 using agroinfection-based Potato Virus X (PVX) expression vectors caused an HR specifically on *R4* plants but not on *r0* plants. However, the HR was only observed when a signal peptide sequence was included in the construct and not when the mature protein was produced. Apparently, ER modification and/or secretion of Avr4 are required for recognition of Avr4 as avirulence factor. Direct virus inoculation of plants with PVX expressing *PiAvr*4 with and without signal peptide sequence gave the same results as the agroinfection inoculation.

505. The plasma membrane H+-ATPase *LmPMA1* is involved in pathogenicity of *Leptosphaeria maculans* on oilseed rape. E. Remy, M. Meyer, M.H. Balesdent, F. Blaise, M. Chabirand, J.P. Narcy, J. Roux, T. Rouxel INRA, Unité PMDV, Route de Saint Cyr, F-78026 Versailles cedex, France

A collection of 5000 *Agrobacterium*-mediated random insertion transformants of the stem canker fungus, *Leptosphaeria maculans*, has been generated. One of these, m210 is severely affected in pathogenicity whereas it is morphologically similar to the WT isolate in vitro and shows no growth or sporulation defect. It induces a typical hypersensibility reaction on oilseed rape leaves and cannot colonize the stem. Electron microscopy experiments further suggested that m210 is depleted in its germination ability on oilseed rape leaf surface. The T-DNA was inserted 274 bp upstream the start codon of one ORF homologous to the highly conserved fungal gene *PMA1*, which encodes the predominant and essential plasma membrane H+-ATPase. Quantitative RT-PCR showed that *LmPMA1* is expressed constitutively at a high level both *in vitro* (germinating conidia, mycelia) and *in planta*, whereas the T-DNA insertion induced a 50% reduction of expression of *LmPMA1* in all tested conditions. Complementation experiments further confirmed that this gene is responsible for the m210 phenotype. The basic function of plasma membrane H+-ATPase is to create an electrochemical proton gradient for (i) uptake of nutrients and (ii) regulation of the intracellular pH. Its expression is under the control of environmental pH and morphogenic development. However, no modification of *LmPMA1* expression levels were observed when investigating these two factors in *L. maculans*, although m210 was affected in its tolerance to various environmental pH. We suggest that the separation of transcriptional regulation boxes from the gene start by the T-DNA insertion led to a deregulation of the expression in m210.

506. Monitoring the induction of trichothecene mycotoxins of *Fusarium graminearum* by using GFP during wheat head infection and in culture. Maier, F.J., S. Jaeger, M. Boenisch, and W. Schaefer, University of Hamburg, Biocenter Klein Flottbek, Ohnhorststr. 18, Hamburg, Germany

The fungal pathogen *F. graminearum* is the most common causal agent of Fusarium head blight (FHB) of small grain cereals and of cob rot of maize. The threat posed by this fungus is due to yield decreases and mycotoxin contamination. It has been shown that trichothecenes influence virulence of *F. graminearum* in a highly complex manner, which is strongly host as well as moderately chemotype specific (Maier et al. 2006). To evaluate the induction of the trichothecenes, 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. Now we are able to monitor the induction of the trichothecene pathway under real time conditions with a cellular resolution. Furthermore, the method needs no time consuming and expensive toxin measurement. By monitoring gfp expression, we evalute the performance of the trichothecene pathway: - during infection of wheat - in culture with plant derived substances, fungicides and miscellaneous chemicals in 96 well plates The results of the current research will be presented. Maier et al. 2006. Involvement of trichothecenes in fusarioses of wheat, barley, and maize evaluated by gene disruption of the trichodiene synthase (Tri5) gene in three field isolates of different chemotype and aggressiveness. Mol. Plant Pathol. 7:449-461.

507. Constitutive expression of the secreted lipase Fgl1 partly restores pathogenicity in apathogenic *Fusarium graminearum* Gpmk1 MAP kinase disruption mutants. Salomon, S., C.A. Voigt, A. Gácser, N.J. Jenczmionka, C. Kröger, S. Frerichmann, and W., Schäfer. University of Hamburg, Biocenter Klein Flottbek, Ohnhorststr. 18, Hamburg, Germany

Mitogen activated protein (MAP) kinases regulate virulence in several pathogenic fungi e.g. *Claviceps purpurea*, *Cochliobolus heterostrophus*, *Magnaporthe grisea*, and *Fusarium graminearum*. Up to now it is unclear which virulence factors are regulated via this signal transduction pathway. We reported that Gpmk1 MAP kinase disruption mutants of *F. graminearum* are apathogenic and cannot infect wheat spikes (Jenczmionka et al., 2003, Curr. Genet.). Additionally, we showed that Gpmk1 MAP kinase disruption mutants exhibit an altered induction of several hydrolytic enzymes (Jenczmionka and Schäfer (2005, Curr. Genet.), among them the secreted lipase FGL1. This lipase is a major virulence factor of *F. graminearum* (Voigt et al., 2005, Plant J.). Here, we show the regulation of the FGL1 gene in dependence on the Gpmk1 MAP kinase in culture and during plant infections. We constructed Gpmk1 MAP kinase deficient *F. graminearum* strains that express the FGL1 gene additionally under the control of the *Aspergillus nidulans* glyceraldehyde-3-phosphate dehydrogenase (gpd) promoter to examine the influence of expression control of the

virulence gene FGL1 by Gpmk1 during wheat infections. These strains are not longer apathogenic on wheat, but in contrast to the wild type strain, they cannot spread out through the entire spike.

508. Improving Maize and Sorghum breeding to turcicum leaf blight in Uganda by fungal population genetics and plant genomics. Tom Martin, Christina Dixelius. Department of Plant Biology and Forest Genetics, Box 7080, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Turcicum leaf blight (TLB) caused by the fungal pathogen *Exserohilum turcicum* is a significant factor contributing to major maize (*Zea mays*) and sorghum (*Sorghum bicolor*) yield losses in sub-Saharan Africa. Population studies can be a powerful tool in order to further understand the variation of the pathogen. *E. turcicum* isolates derived from sorghum and maize hosts will be assessed using AFLP in order to determine population structure and variation in Uganda. Plant molecular defense responses to *E. turcicum* will be analysed. mRNA extractions from interacting fungal and plant cells using laser microdissection will be used in microarrays containing ESTs from maize and sorghum. This will identify up and down regulation of gene expression during pathogen attack thus indicating defense response. Candidate genes will be investigated in order to identify novel resistance genes. Molecular characterization of resistance and polygenic resistance to TLB will lead to the development of markers to support marker assisted breeding programs in East Africa.

509. HOG-like MAP kinase of *Fusarium graminearum* regulates osmotic stress and virulence. Cornelia M. Gregel, Nicole J. Jenczmionka , Wilhelm Schäfer, and Siegfried Salomon. University of Hamburg, Biocenter Klein Flottbek, Ohnhorststr. 18, Hamburg, Germany.

In the course of evolution eukaryotic cells developed a complex network of signal transduction mechanisms to respond to environmental changes. Mitogen-activated protein (MAP) kinases are frequently integrated in these pathways to induce the phosphorylation of many proteins with regulatory functions resulting in a plethora of cellular consequences as proliferation, differentiation, cell cycle arrest, and apoptosis. MAP kinases are ubiquitous enzymes, e.g. in the yeast *Saccharomyces cerevisiae* five MAP kinases are identified that modulate cell growth, differentiation, and stress response. Analysis of the sequenced *Fusarium* genome revealed, like in *Magnaporthe grisea*, the existence of only three conserved MAP kinase genes. Two previously identified enzymes (MGV1 (Hou et al., 2002, Mol Plant Microbe Interact.) and Gpmk1 (Jenczmionka et al., 2003, Curr. Genet.)) are involved in the regulation of mating, hyphal growth, pathogenicity, conidation, and nutrient sensing. We report on the characterization of the third MAP kinase of *F. graminearum*, GOV1, that shows a high homology to the well-characterized yeast protein Hog1, which is implicated in osmotic stress signalling and osmoadaptation. To analyze the function of this Osmo-MAP kinase we generated disruption mutants. The involvement of this MAP kinase in the response to osmolarity stress, viability, conidiation, and pathogenicity will be presented and discussed.

510. Exploring potato microRNA and siRNA in order to mediate resistance to *Phytophthora infestans* : novel approaches to the late blight disease control in potato. Ramesh Raju Vetukuri[1], Christina Dixelius[1], Eugene Savenkov[1]. [1]Department of Plant Biology and Forest Genetics, Box 7080, Swedish University of Agricultural Sciences, Uppsala, Sweden.

The devastating potato late blight is caused by the pathogen *Phytophthora infestans*. As a first step to develop disease resistance, we are in the process of picking out the most suitable *P. infestans* genes as targets for a miRNA- inactivation approach. Functional analysis of virulence in P. infestans revealed several candidate genes. Recently, it has been shown that a G-protein alpha-subunit (encoded by the Pigpa1 gene) plays diverse roles in pathogenesis-related development of *P. infestans* (Latijnhouwers et al., 2004). To obtain resistance to *P. infestans*, we will design miRNA and siRNA constructs to specifically target *P. infestans* candidate virulence genes, namely conservative regions of the Pigpa1 gene and the RxLR motif. On the other hand, several miRNA and siRNA constructs will be designed using a potato miRNA precursor as a backbone. These constructs will target several genes in multi-gene families of the pathogen.

511. The YSST screen identifies secreted proteins of the corn pathogen *Colletotrichum graminicola.* Jorrit-Jan Krijger, Ralf Horbach, Holger B. Deising and Stefan Wirsel; Martin-Luther-Universität, Institut für Agrar- und Ernährungswissenschaften, Halle, Germany;

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The hemibiotroph *Colletotrichum graminicola* is the causal agent of stem rot and leaf anthracnose on its host *Zea mays*. Following germination and penetration of epidermal cells, the fungus enters a short biotrophic phase. Thereafter it switches to the necrotrophic phase which is characterized by hyphal proliferation and killing of the host tissue. Macroscopically this gives rise to leaf anthracnose in which acervuli containing conidia develop. During both phases, secreted proteins may play important roles to establish the interaction. Our interest is focused on proteins that are

involved in suppression of host defense and in reprogramming of host metabolism. To identify genes encoding such proteins, we employ the Yeast Secretion Signal Trap (YSST) in *Saccharomyces cerevisiae* that takes advantage of a 5´-truncated invertase gene on a vector to identify cDNAs carrying a start codon that is followed by a signal sequence. A YSST-cDNA-library was constructed from RNA extracted from *in vitro*-grown mycelium that was induced with corn leaf-extract. To date, we identified 28 unigenes, about one-third of which encode enzymes, mostly hydrolases, and two-thirds encode non-enzymatic proteins. Several of the latter show homology to proteins of unknown function from other phytopathogenic Ascomycota that were annotated in genome projects. RT-PCR experiments indicate changes in expression of some of these genes during progression through the growth phases *in planta*.

512. New insight into antifungal response pathways from a *Candida albicans* protein kinase mutant set. Jill R. Blankenship, Saranna Fanning, Jessica J. Hamaker, and Aaron P. Mitchell. Department of Microbiology, Columbia University Medical Center, New York, NY 10032

Environmental response pathways of pathogens have proven time and again to be critical for infection; the ability to survive macrophage assault or persist during drug therapy requires adaptation to diverse environmental changes. Protein kinases (PKs) play critical roles in environmental response pathways in all organisms. This rationale prompted us to focus on PK genes of the fungal pathogen *C. albicans* as an entry point into dissection of key environmental response pathways. Of 115 predicted PK genes in the genome, we have made insertion mutants for 68. Another 34 may be essential, although our "UAU1" mutagenesis methodology limits the strength of this conclusion. We have focused on the response of *C. albicans* to the cell wall inhibitor caspofungin, a clinically successful antifungal that inhibits glucan synthase activity. Our analysis has led to two unexpected conclusions. First, the highly conserved protein kinase Ire1 is required for the response to caspofungin. Ire1 activates the unfolded protein response to counteract stress in the endoplasmic reticulum, and we propose that caspofungin induces the relevant stress through induction of cell wall protein synthesis. Second, our indication thus far is that Ire1 may be essential for *C. albicans* viability: an insertion near the 3' end of the ORF permits viability, but insertions elsewhere cannot be constructed. Independent verification of this conclusion will suggest that the secretory system is particularly taxed in this organism even under normal laboratory growth conditions.

513. Antifungal Targeting of Signaling in Dermatophytes (*Trichophyton rubrum*). Nalu TA Peres[1], Henrique CS Silveira[1], Nilce M Martinez-Rossi[1] and Rolf A Prade[2], [1]Department of Genetics, Ribeirão Preto School of Medicine, University of São Paulo, and [2]Department of Microbiology and Molecular Genetics, Oklahoma State University E-mail: prade@okstate.edu

Dermatophytes such as *Trichophyton rubrum* select proteins as the preferred carbon and nitrogen source and polar growth is established at the expense of a delicate osmotic balance. During vegetative growth hyphal-tips are swollen and branching is delayed, which is easily recuperated by adding as little as 25 mM of sodium chloride. Antifungal drugs that interfere with osmotic signaling, e.g., ambruticin, enhance hyphal-tip swelling, resulting in rupture of cell walls and membranes with leakage of cell contents leading to cell death. Here we describe a series of ambruticin resistant mutants with a pleiotropic phenotype such as enhanced conidiation, altered pigmentation and modified vegetative growth rates. We also characterize the molecular structure of antifungal targets and its relation to pathogenicity. The antifungal ambruticin interferes with osmotic signaling, turning a non-essential target into a lethal activity.

514. Ecological niche modeling of *Coccidioides sp.* in Western North American deserts. R. C. Baptista-Rosas[1], A. Hinojosa[2] and M. Riquelme[3]. [1]Arid Land Ecosystem Management, School of Sciences, Autonomous University of Baja California (UABC); [2]Geographic Information Systems and Remote Perception Laboratory and [3] Department of Microbiology, Center for Scientific Research and Higher Education of Ensenada (CICESE), Mexico.

Coccidioidomycosis is an endemic infectious disease in Western North American deserts caused by the dimorphic ascomycete *Coccidioides sp.* Eventhough there has been an increase in the reported number of cases in the last years, scarce positive isolations had been obtained from soil samples in endemic areas for the disease. This low correlation between epidemiological and environmental data prompted us to better characterize the fundamental ecological niche of this important fungal pathogen. By using a combination of environmental variables and geospatially referenced points, where positive isolations had been obtained in Southern California and Arizona (U. S.) and Sonora (Mexico), we have applied Genetic Algorithm for Rule Set Production (GARP) and Geographical Information Systems (GIS) to characterize the most likely ecological conditions favorable for the presence of the fungus. This model based on environmental variables, allowed us to identify hotspots for the presence of the fungus in areas of Southern California, Arizona, Texas, Baja California and northern Mexico. Whereas an alternative model based on bioclimatic variables gave us much broader probable distribution areas. We have overlapped the hotspots obtained with the environmental model with the available epidemiological information and have found a high match. Our model suggests that the most probable fundamental ecological niche for *Coccidioides sp.* is found in the arid lands of the North American deserts and provides

the methodological basis to further characterize the realized ecological niche of *Coccidioides sp.* which would ultimately contribute to design smart field sampling strategies.

515. Clp1 is required for in planta proliferation of *Ustilago maydis.* Kai Heimel, Mario Scherer and Jörg Kämper. MPI for terrestrial Microbiology, Marburg, Germany

In the phytopathogenic fungus *Ustilago maydis*, pathogenic development is controlled by a heterodimer of the two homeodomain proteins bE and bW encoded by the *b*-mating type locus. The infection process is closely interconnected with cell cycle control. Formation of the b-heterodimer leads to a G2 arrest of the dikaryotic filament prior to plant infection that is only released after the fungus has penetrated the plant. We have identified clp1 as a *b*-regulated gene that is required for pathogenic development of *U. maydis*. In *clp1* mutant strains, dikaryotic filaments penetrate the plant cuticle, but development is stalled before the first mitotic division, and clamp-like structures that are required for nuclear distribution are not formed. Using the Y2H system, we have identified bW, Rbf1 and the so far unknown bZip transcription factor Cib1 (Clp1 interacting bZIP) as putatively Clp1-interacting proteins. Rbf1 is a zinc finger transcription factor required for the regulation of the majority of *b*-dependent genes; similar to bE/bW, expression of rbf1 leads to a cell cycle arrest. Deletion of *cib1* leads to a phenocopy of the delta-clp1 phenotype. Induced expression of *clp1* strongly interferes with *b*-dependent gene regulation, blocks *b*-dependent filament formation and *b*-dependent cell cycle arrest. These findings place Clp1 in the centre of the regulatory circuits that lead to the establishment of the biotrophic growth-phase and suggest a putative function for Clp1 as a transcriptional modulator, required for cell-cycle release and proliferation in planta.

516. Detection of low ammonium levels and pathogenicity of *Botrytis cinerea*. Martín-Domínguez, R.[1], Perlin, M.H.[2], Eslava, A.P.[1] and Benito, E.P.[1]. [1] CIALE. Universidad de Salamanca. 37007 Salamanca. Spain. [2] Dept. of Biology. University of Louisville, Louisville, KY 40292, USA.

The activation of the infection mechanisms of a phytopathogenic fungus depends on the recognition of a signal that the fungus perceives and that informs it about the presence of a susceptible host. Given the wide host range of *B. cinerea* and its necrotrophic nature, it is possible to presume that the initial signal recognized by this pathogen might be a non-specific, general signal. The detection of essential nutrients availability may constitute a signal of this type. Our main interest is to analyze the ability of *B. cinerea* to detect the ammonium availability as a nitrogen source and to investigate the role of this signal to trigger the activation of its development and pathogenicity mechanisms. The ammonium transport into the fungal cells is carried out by a membrane transporter protein family, the methylammonium permeases. Our group has identified three genes encoding methylammonium permeases in this pathogen. These three genes show different and specific expression patterns during fungal development in minimal medium supplemented with 1 mM ammonium sulphate. We are extending now the analysis of the gene expression patterns during saprophytic and *in planta* growth and on the generation and characterization of mutants altered in each of these three genes. This work is being supported by grant SA06/2005 from ITACyL (Spain)

517. Microarrays meet pathogenicity: Unravelling the secrets of pathogenic development in *Ustilago maydis.* M. Vraneš*, M. Scherer and J. Kämper. Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch- Str., D-35043 Marburg, Germany; vranes@mpi-marburg.mpg.de

In the corn smut fungus *Ustilago maydis*, pathogenic development is strictly dependent on the complex of two homeodomain transcription factors bE and bW. To get insight into the processes that precede plant infection, we performed microarray analysis of *U. maydis* cells grown on the plant surface, comparing a pathogenic strain carrying an active bE/bW heterodimer with a non-pathogenic wild type strain. Four of the b-induced genes on the plant surface encode putative transcription factors. Of particular interest is Biz1 [1], a C2H2 zinc finger protein. Delta *biz1* cells are severely affected in appressoria formation and plant penetration. Hyphae which succeed in penetrating the plant epidermis arrest their growth directly after penetration. Biz1 is involved in the regulation of about 30% of all genes induced on the plant surface; for 19 of these genes, Biz1 is both required and sufficient for induction. Systematic deletion analysis of these genes led to the identification of *pst1* and *pst2*, encoding potentially secreted *U. maydis* specific proteins. Delta *pst1/pst2* cells are still able to penetrate the plant surface, but subsequently fail to invade and colonize the plant, resembling the *biz1* deletion phenotype. In contrast to wild type strains, for biz1 as well as for pst1/pst2 deletion strains reactive oxygen species (ROS) can be detected at the site of penetration, suggesting a function in suppression of plant defence for Pst1/Pst2. Furthermore, 76 Biz1 induced genes are differentially regulated during the entire pathogenic development. Thus, Biz1 appears not only to be a regulator for genes required for plant penetration, but also for genes with impact on pathogenicity at later stages. [1] Vraneš et al., Plant Cell Sep;18(9): 2369-2387

518. Distinct roles for intra- and extracellular siderophores during *Aspergillus fumigatus* infection. Markus Schrettl[1], Elaine Bignell[2], Claudia Kragl[1], Yasmina Sabiha[1], Martin Eisendle[1], Herbert N. Arst Jr[2], Kenneth Haynes[2], Hubertus

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The ability to acquire iron *in vivo* is essential for most microbial pathogens. Consistently, a siderophore lacking *Aspergillus fumigatus* mutant, *deltasidA*, is avirulent in a murine model of invasive aspergillosis. We have previously demonstrated that *A. fumigatus* excretes fusarinine C (FSC) and triacetylfusarinine C (TAFC) to capture extracellular iron, and uses ferricrocin (FC) for intracellular hyphal iron storage. Here we show that *A. fumigatus* synthesizes a fourth siderophore, termed hydroxyferricrocin (HFC), employed for intracellular conidial iron storage. Functional characterisation of four genes enabled analysis of the relative contributions of these siderophores to virulence. The conversion of FSC to TAFC is not required for virulence, as evidenced by equivalent virulence of the non acetyl transferase synthesising mutant *deltasidG*, to that of the wild type isolate. However, abrogation of extracellular siderophore biosynthesis, following inactivation of the *sidF*-encoded acyl transferase or the nonribosomal peptide synthase SidD, leads to complete dependence upon reductive iron assimilation (RIA) under iron limiting conditions, and sensitivity to oxidative and nitrosative stress *in vitro*, as well as significantly reduced virulence. Deficiency in intracellular iron storage by inactivation of the nonribosomal peptide synthase SidC leads to delayed germination, sensitivity to oxidative and nitrosative stress and attenuated virulence. Reconstitution of the conidial HFC content partially restored virulence of the *A. fumigatus deltasidA* mutant emphasizing an important role of HFC during the initial phase of infection. This work was supported by the Austrian Science Foundation), the Chronic Granulomatous Disorder Research Trust and the Biotechnological and Biological Sciences Research Council.

519. *In planta* competitive interactions between endophytic isolates of *Fusarium verticillioides* and a maize fungal pathogen, *Ustilago maydis* result in reduced smut disease incidence. Keunsub Lee[1], Georgiana May[1, 2]. University of Minnesota Twin Cities [1]Plant Biological Sciences Graduate Program. [1]Department of Ecology, Evolution & Behavior, Minnesota, United States

An ascomycete fungus *Fusarium verticillioides* is widespread throughout the world and often causes symptomless infections. We have investigated the effects of endophytic *F. verticillioides* on the fitness of maize and a fungal pathogen, *Ustilago maydis* using *in vitro* and *in planta* experiments. On PDA plates, *F. verticillioides* isolates grew over the colonies of *U. maydis* sporidia, suggesting a possible antagonism against the pathogen. *In planta* interaction experiments were conducted in a greenhouse with multi-factorial design: 2 *F. verticillioides* isolates X 2 *U. maydis* isolate pairs X 3 dates of *F. verticillioides* inoculation. The mixture of two mating compatible sporidia of *U. maydis* was inoculated onto the whorl of 9-day-old maize seedlings and the spore suspension of *F. verticillioides* was pipetted onto the 7, 9, and 11-day-old maize seedlings. Maize height and smut disease symptoms were measured at 17 and 19 days post planting, respectively. Our data suggested that the endophytic isolates of *F. verticillioides* do not directly enhance maize growth but can reduce smut disease incidence when the two fungal spores were simultaneously inoculated to maize seedlings in the greenhouse condition.

520. Modulation of fungal development and pathogenicity by apoptotic proteins of the Bcl-2 family. Amir Sharon and Sima Barhoom Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel

Proteins of to the Bcl-2 family are key regulators of mitochondria-mediated apoptosis. They are present in metazoan organisms, but absent in plants and fungi. Nevertheless, heterologous expression of anti-apoptotic Bcl-2 protein members can prevent cell death and protect plants from necrotrophs. In yeasts, Bcl-2 proteins affect programmed cell death, and cells expressing anti-apoptotic Bcl-2 members are more stress resistant and have an extended life span. This suggests that despite lack of structural homologs, Bcl-2 proteins might recognize and interact with elements that regulate cell death and development in plants and fungi. To further study the possible role of cell death in fungi, we expressed human Bcl-2 proteins in the plant pathogen Colletotrichum gloeosporioides. Expression of the pro-apoptotic Bax protein lead to cell death with apoptotic characteristics, while cells expressing the anti-apoptotic Bcl-2 protein were protected from Bax-induced cell death. The expression of the Bcl-2 proteins not only affected cell death, but was also associated with a wide range of developmental changes, including alterations in germination, life span, stress resistance, and pathogenicity. Search of a C. gloeosporioides EST database for potential Bcl-2 interacting proteins identified a homolog of the human TCTP gene. The CgTCTP gene was isolated and the protein showed strong and specific interaction with Bcl-2 in a yeast two hybrid interaction assay. Strains over expressing the CgTCTP protein had only part of the phenotypes displayed by Bcl-2 expressing strains, suggesting that Bcl-2 might interact with several different proteins to exert its full effects on the fungus. Our findings show that programmed cell death-associated machinery, which can be affected by Bcl-2 proteins, regulates fungal development and adaptation to different environments.

521. Classification of resistance genes from wild *Solanum* species based on responses to RXLR effectors of *Phytophthora infestans*. Nicolas Champouret[1], Carolyn Young[2], Minkyoung Lee[2], Sophien Kamoun[2], Evert Jacobsen[1], Richard Visser[1]

and Vivianne Vleeshouwers[1]. [1]Department of Plant Science – Laboratory of Plant Breeding, Wageningen, The Netherlands. [2]Ohio State University, OARDC, Department of Plant Pathology, Wooster, OH, USA.

The causal agent of potato late blight *Phytophthora infestans* possesses numerous genes encoding extracellular effector proteins. One class of these secreted proteins contains a host cell targeting (HCT) motif centered on an RXLR core, which is a well-studied motif in effectors of the human malaria parasite *Plasmodium falciparum*. RXLR effectors are thought to be translocated inside the plant cell as they are inside the erythrocyte cell. In the plant cell they can interact with the intracellular NBS-LRR Resistance proteins, the most common class of R proteins effective to oomycetes. In addition to the known isolated R genes from *S. demissum* and *S. bulbocastanum*, other R genes are identified in various other wild *Solanum* species. In this study, we test some of these plants in a transient expression system based on *Agrobacterium tumefaciens* and potato virus X (PVX) expressing cDNAs of RXLR candidate effectors of *P. infestans*. A number of specific responses to various RXLR effectors were identified in these wild and cultivated *Solanum*. Classification of different resistances could be made based on the recognition pattern of the *P. infestans* RXLR effectors. The aim is to comprise a catalogue of RXLR effectors useful for functional profiling of other R-genes in wild *Solanum*.

522. Two-component signal transduction system of rice blast fungus is involved in osmotic stress response, fungicide action, and pathogenicity. Takayuki Motoyama[1], Masumi Morita[2], Naoko Ochiai[1], Ron Usami[2], and Toshiaki Kudo[1]. [1]RIKEN, Wako, Saitama, Japan. [2] Toyo University, Kawagoe, Saitama, Japan.

Rice blast fungus (*Magnaporthe grisea*) has ten histidine kinases (HK), one histidine-containing phosphotransfer protein (HPt), and three response regulators (RR) as putative components of the two-component signal transduction system (TCS). We previously constructed a disruptant of an HK gene (*HIK1*) of this fungus and found that Hik1 is involved in osmotic stress response and fungicide action. Here, we constructed knockout mutants of three putative RR genes (*MgSSK1*, *MgSKN7*, *MgRIM15*) to analyze the roles of TCS in environmental adaptation and pathogenicity. Delta*Mgssk1* strain showed increased sensitivity to high osmolarity, decreased sensitivity to fludioxonil, and decreased pathogenicity to rice plants. In contrast, Delta*Mgskn7* and Delta*Mgrim15* strains showed no such phenotypes. To analyze downstream signal pathway of MgSsk1, we examined phosphorylation (activation) of Osm1 MAP kinase. Delta*Mgskn7*, Delta*Mgrim15*, and wild-type strains could activate Osm1 in response to high osmolarity and fludioxonil. In contrast, Delta*Mgssk1* strain could not activate Osm1, suggesting that MgSsk1 is an upstream regulator of Osm1. These results indicate that TCS of the rice blast fungus is involved in osmotic stress response, fungicide action, and pathogenicity.

523. Cell-specific Gene Expression of Human - *Trichophyton rubrum* Interaction. Nalu TA Peres[1], Diana E Gras[1], Nilce M Martinez-Rossi[1] and Rolf A Prade[2]. [1]Department of Genetics, Ribeirão Preto School of Medicine, [2]University of São Paulo and Department of Microbiology and Molecular Genetics, Oklahoma State University E-mail: nalu@usp.br

***Trichophyton rubrum* is a medically relevant mold, widely encountered in superficial cutaneous infections. Although these infections are generally restricted to the stratum corneum of the skin, immunocompromised patients often develop deep local invasions with multivisceral dissemination that can result in death. We established an "in situ" human fungal cell interaction system to study dermatophyte-host interactions. We established the adhesion of *T. rubrum* microconidia to human epidermal skin-cells, germination and production of hyphae. We employed subtractive suppressive hybridization (SSH) to identify genes specifically expressed by *T. rubrum* during "in situ" interaction with human skin. Ambruticin is a new antifungal compound, which targets the ability of the fungus to adapt its osmoticum, accumulates large amounts of glycerol and dies. We describe the effect of ambruticin on human fungal cell interactions and its effect of gene expression.**

524. Transcriptional responses in the fungus *Monacrosporium haptotylum* during the infection of the nematode *Caenorhabditis elegans.* Margareta Tholander, Csaba Fekete, Dag Ahrén, Eva Friman, Balaji Rajashekar, Tomas Johansson and Anders Tunlid Department of Microbial Ecology, Lund University, Ecology Building, SE-223 62 Lund, Sweden. E-mail: Anders.Tunlid@mbioekol.lu.se.

Nematode-trapping fungi infects their hosts in a sequence of events including adhesion of the traps to the host surface, penetration followed by digestion and growth on the host tissue. To identify proteins that could be involved in the killing of the nematodes, we are identifying and characterizing transcripts that are uniquely expressed during the penetration of captured nematodes. The global patterns of gene expression were followed in the fungus *M. haptotylum* during the infection of the nematode *C. elegans* . RNA was isolated during different stages of the infection and hybridized to a cDNA array containing 2684 fungal and 581 worm gene reporters. The different stages of infection were associated with dramatic shifts in the fungal transcriptome. Among the regulated genes, we identified a cluster of 372 ESTs that were transiently and specifically up-regulated during penetration. This cohort had a high number (293) of sequences displaying no significant similarity to genes in other organisms. DNA sequencing showed that these orphans contained transcripts that translate to presumably full-length proteins, but also numerous transcripts with disabled ORFs (frameshifts and premature stop codons). The function of these early expressed proteins and non-coding RNAs are not yet known.

525. *In Planta* Secretion of Effector Proteins by the Rice Blast Fungus *Magnaporthe grisea*. Chang Hyun Khang[1], Romain Berruyer[1], Sook-Young Park[2], Prasanna Kankanala[1], Kirk Czymmek[3], Seogchan Kang[2], and Barbara Valent[1]. [1] Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA. [2] Department of Plant Pathology, The Pennsylvania State Univerity, University Park, Pennsylvania, USA. [3] Department of Biological Sciences, University of Delaware, Newark, Delaware, USA.

The rice blast fungus *Magnaporthe grisea* invades and grows within living plant cells to establish a successful infection. AVR-Pita, one of the effector proteins of this fungus, is only expressed during infection, and it appears to interact with the resistance protein Pi-ta inside the rice cell. Contrary to the well-studied bacterial type III secretion machinery by which bacterial effector proteins are injected into the plant cells, it is unknown how fungal effector proteins are secreted and cross the plant membrane to gain access to the plant cytoplasm. To visualize secretion of AVR-Pita protein into invaded rice cells and to define sequences required for its secretion, we generated fungal transformants expressing various portions of the AVR-Pita protein fused to green fluorescent protein. The fusion proteins accumulated in novel membrane-rich structures in invaded rice as well as in barley. These structures are only seen as the fungus grows *in planta* and they do not contain fungal cytoplasm. Other effector proteins, PWL1 and PWL2, were also localized in these structures. This localization requires the N-terminal signal peptide of these effector proteins. We hypothesize that these specialized structures are involved in delivering fungal effector proteins inside plant cells.

526. The coiled-coil protein-binding motif in Fsr1 is essential for maize stalk rot virulence in *Fusarium verticillioides*. Yoshimi Yamamura and Won-Bo Shim, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX77843-2132

***Fusarium verticillioides* is one of the key pathogens of maize stalk rot disease. To date, however, clear understanding of stalk rot pathogenesis is lacking. Previously, we identified a gene in *F. verticillioides*, designated *FSR1*, that plays a key role in fungal virulence and female fertility. The putative Fsr1 protein, a polypeptide of 837 amino acids, contains multiple protein-binding domains, namely a caveolin-binding domain and a coiled-coil structure at the N-terminus and WD40 repeat domain at the C-terminus. Fsr1 shares significant similarity to a family of animal proteins that play a critical role in cellular mechanisms that regulate a variety of developmental processes. Significantly, *FSR1* function is conserved in *F. graminearum*, where it also plays a direct role in pathogenesis. The objective of this study was to determine the functional role of the putative protein binding motifs in Fsr1 associated with fungal virulence. First, we complemented the *FSR1* knock-out (*fsr1*) strain with mutated versions of the *FSR1* gene. Our data demonstrated that the Fsr1 C-terminal WD40 repeat domain is dispensable for vegetative growth and maize stalk rot virulence. This strongly suggests that a protein binding motif(s) located in the N-terminus is required for stalk rot virulence. Currently, we are testing whether the caveolin-domain or the coiled-coil structure is essential for virulence in *F. verticillioides*. In addition to motif characterization study, we are performing a yeast two-hybrid screen using the N-terminus sequence as a bait to isolate proteins that bind to Fsr1 *in vivo*.**

527. Tagging pathogenicity genes in *Fusarium graminearum* using the transposon system *mimp/impala*. Marie Dufresne[1,*] Theo van der Lee[2,*] Sarrah Ben M'Barek[1,2] Taiguo Liu[1] Cees Waalwijk[2] Wenwei Zhang[2] Gert H.J. Kema[2] and Marie-Josée Daboussi[1]. [1] Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France [2] Plant Research International B.V., P.O. Box 16, 6500 AA, Wageningen, The Netherlands [*]equal contribution

The number of predicted genes present in the genome of *Fusarium graminearum* is estimated to be around 14,000. For many genes the function is yet unknown and consequently there is a need for a high-throughput method for functional analyses of genes. We applied a transposon mutagenesis strategy using a mite element (*mimp1*) activated by a transposase (*impala*). Previously we have shown that the double component system *mimp1/impala* transposase is fully functional in *F. graminearum* (Dufresne et al., 2006). Transposition characteristics and high reinsertion frequency were found to be the same as in the original host species, *F. oxysporum*, allowing the application of this double component system for the generation of large transposon mutant collections. We transformed the double component system into *F. graminearum* strain FG820, selected 100 revertants and determined the sequence flanking the *mimp1* reinsertion sites using TAIL-PCR and the *F. graminearum* genome sequence. In 53% of the isolates *mimp1* reinserted close to or in genes. Subsequently, a pilot collection of around 300 revertants from the same transformant (FG820-6-11) was screened for growth on a large set of media and for pathogenicity on wheat. Several revertants with altered phenotypes were identified and in one of them *mimp1* reinserted into an ORF encoding a transcription factor (FG820-6-11-r112). The relationship between *mimp1* insertion and the mutant phenotype is currently investigated by functional complementation. Our results indicate that this novel double component transposon system is a powerful mutagenesis tool for high-throughput analysis of *F. graminearum* and potentially other ascomycete fungi.

Dufresne M*, Hua-Van A*, Abdel Wahab H, Ben M'Barek S, Vasnier C, Teysset L, Kema G and Daboussi MJ (2006) Transposition of a fungal MITE through the action of a Tc1-like transposase. Accepted for publication in Genetics (*equal contribution).

528. Expression analysis of the *Botrytis cinerea* flavohemoglobin coding gene (*Bcfhg1*). Juan L Turrion-Gomez and Ernesto P Benito. CIALE, Dept. of Microbiology and Genetics, University of Salamanca, 37007, Salamanca, Spain. juanturrion@usal.es

Flavohemoglobins constitute a group of proteins involved in responding to nitric oxide (NO) and nitrosative stress. Hence, a flavohemoglobin encoding gene from *B. cinerea* was isolated (*Bcfhg1*). For expression analysis, an experimental setup was developed to expose *B. cinerea* to NO *in vitro* by using the NO donor DETA (Diethylenetriamine, Sigma) at 0.125, 0.25 and 0.5 mM. For *in planta* expression analysis, infected tomato leaves were collected at 8, 12, 16, 32, 48, 72, 96 and 120 hours post inoculation (hpi). Northern analysis demonstrated a basal level of expression of *Bcfhg1 in vitro* in the absence of NO which increases between 4 and 6 hours of incubation and declines after 8 hours of incubation. After exposure to 0.5-1.4 uM of NO the level of expression increased very quickly (5 minutes), reaching a maximum 15 minutes after induction with DETA. Interestingly, in terms of induction of expression of *Bcfhg1*, germinating conidia (grown during 4 hours in liquid medium before exposure) appeared to be more sensitive to NO than mycelium grown during 12 hours in liquid medium. *In planta* expression was maxima 8 hpi and significantly decreased after 12 hpi. From 12 to 120 hpi the expression was constant and about 4 times lower than at 8 hpi. Taken together our observations indicate that expression of *Bcfhg1* follows a similar pattern during saprophytic growth and during *in planta* growth and that it is enhanced after exposure to NO. This work is supported by grant AGL2005-06049.

529. Pathological and genetic characteristics of *Fusarium oxysporum* isolates from runner beans (*Phaseolus coccineus* L.). J.J. de Vega*, A. Stefanska, R. Martín-Domínguez, M.A. García-Sánchez, B. Ramos, A.P. Eslava and J.M. Díaz-Mínguez Departamento de Microbiología y Genética. Centro Hispano-Luso de Investigaciones Agrarias (CIALE). University of Salamanca. 37007 - Salamanca, SPAIN. Phone/Fax: +34.923.294663 *email: josejavier@usal.es

The *Fusarium oxysporum* f.sp. *phaseoli* isolates used in this study were recovered from diseased plants of runner bean (*Phaseolus coccineus* L.) in the area of El Barco de Avila in West-Central Spain. The isolates were characterized by means of pathogenicity tests, vegetative compatibility groups, polymorphisms of the intergenic-spacer (IGS) region of ribosomal DNA and distribution of mating-type (MAT) idiomorphs. In previous works we had defined two virulent groups (highly virulent and weakly virulent) for *Fusarium oxysporum* f.sp. *phaseoli* strains isolated from common bean (*Phaseolus vulgaris* L.). However, the pathogenicity assays developed on *Phaseolus coccineus* allow the distribution of the new isolates into three groups because of a new group of super virulent strains. Previous works reported that highly and weakly virulent strains were diferential for the presence of *ftf1* gene which is involved in early stages of the infection. We have performed PCR and Southern experiments looking for diferential results among super, highly and weakly virulent strains. The strains here analyzed belong to new VCGs, namely VCG 169, 1610, 1611 and 161. The new characterized strains belongs to IGS-A group (the IGS group of the pathogenic strains isolated from *Phaseolus vulgaris*) but also to IGS-B2 group. Eventually, we have found only the MAT1-2 idiomorph among these Spanish *Fusarium oxysporum* f.sp. *phaseoli* strains.

530. Evolution in complex symbioses. Georgiana May, Keunsub Lee, Peter Voth. Department of Ecology, Evolution and Behavior, U. Minnesota, St. Paul, MN, gmay@umn.edu

In this work, we address the question of how symbioses such as host/pathogen interactions evolve in a broader ecological context. Are the molecular mechanisms of interaction the consequence of pairwise species' evolutionary interactions or the result of continual compromises in the context of simultaneous multiple species' interactions? Information will be presented for interactions among the multiple fungal symbionts of maize and for interactions of those fungal species with the host, maize. We demonstrate that outcomes of the interactions are not predictable from individual pairwise interactions. Interactions of two fungal symbionts simultaneously with their host species can differ qualitatively and quantitatively from the interactions of either fungal symbiont with the host species alone. The results lend insight into conditions under which pathogens may exhibit less aggressive to their hosts and raise questions for the 'arms race' evolutionary models for pathogens and their hosts.

531. Vacuolar H^+-ATPase plays a role in the pathogenicity of *Magnaporthe oryzae*. Melania F. Betts, Natalia Galadima, Lei Li, M. Alejandra Mandel, Marc J. Orbach. University of Arizona, Tucson, AZ 85721

***Magnaporthe oryzae* is a filamentous ascomycete responsible for causing rice blast disease. Infection occurs when *M. oryzae* differentiates a specialized infection cell, the appressorium, which utilizes mechanical force to enter host cells. This mechanical force is generated via an increase in turgor pressure via elevated glycerol levels in the appressorium. During**

generation of a large collection of T-DNA insertion lines, we screened strains for visible defects in appressorium development using an *in vitro* assay. Screening of 12,000 strains resulted in the identification of 135 mutants with apparent defects either in morphology or appressorium development. These strains were then analyzed for penetration in an onion epidermis assay and screened for pathogenicity on rice plants. This resulted in the identification of 112 strains that were defective in pathogenicity. Inverse PCR was used to identify the putative defective gene in several of these mutants. We focused on one mutant with reduced pathogenicity and appressorium function that was due to an insertion in the promoter region of the *M. oryzae* vacuolar type ATPase subunit c`` gene. We designate this gene *MVA1*. Genetic complementation and targeted disruption of *MVA1* confirmed its role in pathogenicity. The *mva1* insertion line was found to be impaired in conidiation, host penetration and the generation of turgor pressure in the appressorium. Here, we report MVA1 spatial and temporal expression analyses, and efforts towards its functional characterization.

532.Withdrawn

533. Sclerotial mycoparasitism by *Coniothyrium minitans*: Gene expression and function. S. Sreenivasaprasad, S. Muthumeenakshi, C. Rogers, M. P. Challen and J. M. Whipps. Warwick HRI, University of Warwick, Wellesbourne, Warwickshire CV35 9EF, UK.

***Coniothyrium minitans* colonises and destroys the sclerotia of *Sclerotinia sclerotiorum* in nature. *C. minitans* exhibits an ecologically obligate mode of mycoparasitism as its spores remain dormant in soil and only grow actively in the presence of the sclerotia of *S. sclerotiorum*. Currently, *C. minitans* is exploited as a commercial biocontrol agent, but the molecular mechanisms underlying this specialised host-parasite interaction are poorly understood. Suppression subtractive hybridisation was used to generate a cDNA library enriched for genes up- regulated during sclerotial mycoparasitism. Sequencing and bioinformatic analysis led to the identification of more than 250 unisequences and their assignment to various functional categories. Further, a set of mycoparasitism-deficient *C. minitans* mutants were identified from 4000 transformants generated by insertional mutagenesis. Molecular analysis of some of these mutants enabled the identification of putative genes. Complementation and gene silencing technologies are being tested for functional analysis of some of the key genes. Comparative analysis identified genes associated with key processes such as signalling (GPCRs), host degradation (glycosidases and peptidases), nutrient utilisation (MFS transporters), detoxification and stress response (ABC transporters, ROS scavengers) and DNA repair (*PIF1* helicase) suggesting that *C. minitans* employs specialised host colonisation strategies. Several of these genes are novel to fungal-fungal interactions. This lays the platform for comparative and functional genomics of sclerotial mycoparasitism.**

534. Role of Cst12 in parasitic growth of the human pathogenic fungus *Coccidioides posadasii*. Lei Li[1, 2]. Ellen M. Kellner[1, 2], Lisa F. Shubitz[1, 3], John N. Galgiani[1, 4] and Marc J. Orbach[1, 2]. Valley Fever Center for Excellence[1], Department of Plant Sciences[2], Department of Veterinary Sciences and Microbiology[3], University of Arizona, and Southern Arizona Veteran's Affairs Health Care System[4], Tucson, Arizona 85721, USA

Valley fever is a disease endemic in the southwestern United States as well as Central and South America. It is caused by *Coccidioides spp.*, the dimorphic fungi dwelling in desert soils as filamentous saprobes that reproduce by arthroconidiation. Upon inhalation by mammalian hosts, the arthroconidia initiate parasitic growth in the lung, which involves isotropic cell growth to produce spherules with nuclear division preceding septation, and endosporulation. When endospores disseminate to extrapulmonary sites, the disease may be lethal, especially in patients with suppressed immune systems. Molecular genetic studies of *Coccidioides* have primarily focused on the identification of antigens for potential vaccine development. There has been less focus on *Coccidioides* virulence factors. In this study, we have identified a potential virulence factor Cst12 (for *Coccidioides* Ste12 homolog) that is important for spherule development. Gene replacement mutants were generated for *CST12* in *C. posadasii* strain Silveira. No defects were observed in arthroconidiation, spore germination or hyphal growth. However, during *in vitro* parasitic growth, the spherules produced by the *cst12* mutants failed to mature beyond an early stage of 48 to 72 hours post inoculation when they were about 5µm in diameter. In contrast, Silveira spherules continued to enlarge up to 15µm by 120 hours post inoculation. We hypothesize that either cell division or septation of the developing spherules is impaired during parasitic growth of the *cst12* mutants. Pathogenicity tests of the *cst12* mutants using a murine model are under way to further evaluate the role of Cst12 in the *in vivo* parasitic cycles.

535. Molecular analysis of population diversity and pathogenicity in *Colletotrichum acutatum*. S. Sreenivasaprasad[1], P. Talhinhas[2], S. Muthumeenakshi[1], J. Neves- Martins[2] and H. Oliveira[2]. [1]Warwick HRI, University of Warwick, Wellesbourne, Warwickshire CV35 9EF, UK. [2]Instituto Superior de Agronomia, Tapada da Ajuda, 1349- 017 Lisboa, Portugal.

***Colletotrichum acutatum* is a cosmopolitan pathogen causing anthracnose diseases on a wide range of hosts including citrus, strawberry, olive, peach, almond and lupin. We are using a range of molecular approaches for understanding the**

population diversity and pathogenicity in this pathogen. Phylogenetic analyses of 289 ITS sequences representing global *C. acutatum* populations revealed eight molecular groups A1 - A8 with varying biogeographic association patterns. Based on *ITS*, *tub2* and *his4* sequences, *C. acutatum* isolates from lupins from worldwide locations were genetically homogeneous and formed a distinct group, leading to the designation of this pathogen as a separate species by some authors. On the contrary, *C. acutatum* isolates from olive within Portugal comprised five molecular groups. PCR tests based on ITS and tub2 sequences enabled rapid and reliable diagnosis and differentiation of *C. acutatum* populations as well as detection in asymptomatic olive tissue. *C. acutatum* is known to exhibit different pathogenic strategies on various hosts, but the components regulating these processes are only beginning to be understood. We are using forward genetics through *Agrobacterium*-insertional mutagenesis in *C. acutatum* as well as the *Colletotrichum-Arabidopsis* model system to investigate the pathogenicity lifestyles in Colletotrichum.

536. Jasmonic acid activity and *Fusarium oxysporum* virulence. Andrew Diener. University of California, Los Angeles, CA

Jasmonic acid (JA) is a plant hormone and is typically associated with responses to stress: wounding, herbivory and necrotrophic pathogens. However, JA and JA-related compounds are also produced by some fungi, including the phytopathogen *Fusarium oxysporum*. What metabolism is involved in fungal JA biosynthesis and how JA expression is regulated in a fungus are unknown. To answer these two questions, I am examining mutants of candidate genes of *F. oxysporum* for altered JA activity. Whether fungal derived JA has a role in plant disease is unknown too. Because a necrotrophic lifestyle is frequently attributed to *F. oxysporum*, it seems counterintuitive that JA would promote virulence or disease. To address this question, I am examining the pathogen-host interaction in Fusarium wilt disease of Arabidopsis. We also intend to report the behavior of marked *F. oxysporum* pathogenic isolates, at the microscopic level, in relevant Arabidopsis mutants.

537. Diversity of *Venturia inaequalis* causing Apple and Crabapple Scab epidemics in the Northeastern U.S. Gnana A. Viji, James W. Travis, and Maria M. Jimenez-Gasco. Department of Plant Pathology, The Pennsylvania State University, University Park.

Apple scab caused by *Venturia inaequalis* is the most severe and widespread disease of cultivated apples worldwide. The disease also affects ornamental crabapples from various *Malus* spp. In this study, microsatellite markers developed from European isolates of *V. inaequalis* were used to characterize the genetic diversity of isolates obtained from cultivated apples and ornamental crabapples from various geographical regions in the northeastern U.S. Isolates of the pathogen from both hosts revealed a high level of diversity and displayed up to fourteen alleles per locus. The genetic relationship between *V. inaequalis* populations from apples and crabapples analyzed using Nei's index of genetic distance showed that allelic diversity at each locus tested was not significantly different ($P < 0.05$) among the populations from both hosts or geographic origin. Genetic distance measured as pairwise comparisons using UPGMA based on simple matching coefficient showed a high level of genetic diversity, as expected from a sexually- reproducing organism. No correlation with host of origin was identified. The number of migrants as determined by POPGENE was high (n=16) indicating a constant gene flow among the isolates from both hosts. Pathogenicity tests on apple and crabapple indicate that isolates can cross infect regardless of their host of origin. Our results raise the possibility that crabapple populations may serve as reservoirs of the pathogen and the role of crabapple pathogen populations in apple scab epidemics should be considered further for efficient scab management.

538. Fungal tetraspanins: key players in appressorium-mediated penetration of host plants. Karine Lambou [1], Melanie Marguerettaz [1], Anne-Laure Raffin [1], Catherine Sirven [2], Roland Beffa [2], and Marc-Henri Lebrun [1]. [1]UMR2847 CNRS-Bayer Cropscience, Lyon, France [2] Biochemistry Department, Bayer Cropscience, Lyon, France

Fungal plant pathogens rely on a specialised cell called the appressorium to penetrate into host plant. *M. grisea* insertional mutant *punchless* is unable to penetrate into rice and barley leaves, although it forms melanized appressoria. The gene inactivated in *punchless* encodes for a small membrane protein, Pls1, from tetraspanins super-family. In animals, tetraspanins act as molecular adaptators required for the formation of membrane signalling complexes. Pls1 is only expressed in appressoria and mainly localized in vacuoles. *PLS1* deletion has a pleiotropic effect on appressoria including the absence of penetration peg. To identify the cellular functions controlled by Pls1, we used a genome wide array to compare expression profiles of a *pls1f´* mutant to wild type. Most of the 417 genes differentially expressed in *pls1f´* appressoria are up-regulated and encode secreted/membrane proteins and enzymes from secondary metabolism. These up-regulations likely result from a cellular stress induced by the lack of Pls1. A Pth11-GPCR, amino acid transporters and membrane proteins were identified as Pls1 interactants using a split ubiquitin yeast two hybrid assay. These results suggest that Pls1 is involved directly or indirectly in the sorting of appressorial membrane proteins.

539. *Stagonospora nodorum* utilizes multiple proteinaceous host selective toxins which interact with dominant host sensitivity genes in wheat. Timothy L. Friesen[1], Justin D. Faris[1], and Richard P. Oliver[2]. [1]USDA-ARS Cereal Crops

Research Unit, Northern Crop Science Laboratory, 1307 18th Street North, Fargo ND, 58105. [2]Australian Centre for Necrotrophic Fungal Pathogens, Western Australian State Agricultural Biotechnology Centre, Division of Health Science, Murdoch University, Western Australia 6150.

Stagonospora nodorum **has recently been shown to produce multiple proteinaceous host selective toxins that interact either directly or indirectly with dominant sensitivity genes in wheat. ToxA, a proteinaceous host selective toxin originally associated with *Pyrenophora tritici-repentis*, was recently identified in *S. nodorum*. The *ToxA* gene was convincingly shown to have moved from *S. nodorum* to *P. tritici-repentis* by a recent horizontal gene transfer event. SnTox1 has also been identified and shown to be highly associated with disease development. More recently we have identified and characterized two additional toxin interactions for their significance in disease. SnTox2 and SnTox3, which are produced by the majority of North American isolates tested, interact with the host sensitivity genes *Snn2*, located on wheat chromosome 2D, and *Snn3* located on chromosome 5B. SnTox2 is a protein with an approximate size of 6.6 kDa whereas SnTox3 is a protein in the 10 to 30kDa range. By using wheat mapping populations segregating for *Tsn1* (ToxA sensitivity), *Snn2* (Tox2 sensitivity), and *Snn3* (Tox3 sensitivity), disease significance for each toxin sensitivity gene has been shown to account for as much as 60% of the disease phenotype caused by *S. nodorum* isolates producing each toxin. Several additional uncharacterized toxins have also been identified. The accumulation of work on the *S. nodorum* host pathogen system establishes it as a complex inverse gene-for-gene system where multiple host selective toxins interact with dominant host sensitivity gene products to induce disease.**

540. ***Lysobacter enzymogenes*****, a bacterial pathogen that internalizes in fungal cells. R.F. Sullivan[1], J.A. Crouch[1], V. Alla[1], P. Dave[1], E.Chi[1], S. Pinhancos[2], D.Y. Kobayashi[1] and B.I. Hillman[1]. [1]Dept. Plant Biology & Pathology, Rutgers University, New Brunswick, NJ. [2]NJCSTME, Kean University, Union, NJ. Email: raysully@gmail.com.**

The bacterial biocontrol agent ***Lysobacter enzymogenes*** **uses a variety of mechanisms to antagonize and infect fungi, including the production of numerous fungal cell wall degrading enzymes, secondary metabolites, and a type III secretion system. Macroscopic observations indicate *L. enzymogenes* can colonize the mycelia of a vast range of fungal species in a unique, stage-specific manner, where bacterial suspensions are rapidly absorbed into the fungal colony leading to hyphal lysis followed by expansion of the ruptured region. However, while bacterial suspensions have been observed to adsorb into most fungal host colonies tested, clear differences in post-absorption reactions can be observed among different fungal taxa as well as strains of a single species. Microscopic observations using *Magaporthe oryzae* as a host indicate the bacterium is capable of colonizing hyphae both externally and internally. Knockout mutant strains of *L. enzymogenes* C3 identified enzymes and secondary metabolites important for both mycelial colonization and internalization within hyphae. These results indicate the *L. enzymogenes* is an ideal model system to study bacterial pathogen-fungal host interactions.**

541. Withdrawn

542. Identification of Secreted Proteins of ***Botrytis cinerea*** **Associated with Growth on Strawberry. Hind El Mubarek[1], Punit Shah[2], Ron Orlando[2], Gopi K. Podila[1] and Maria R. Davis[1] [1] Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, AL, USA [2] Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA**

Botrytis cinerea **is a filamentous phytopathogenic fungus that causes gray-mold rot of berries, tomatoes, peas and a number of leafy vegetables and fruits with low pH. Secreted proteins are the core component of the degrading lifestyle of filamentous fungi and their pathogenic ability. With an increasing availability of genome sequence data, it is becoming easier to study the role of secreted proteins in the pathology and survival of fungal pathogens. Mass spectrometry (MS/MS) techniques have facilitated the identification of proteins from organisms with little or no genome sequence available. While in cases where genome information is available, MS/MS techniques provide a reverse proteomic analysis by providing a novel view of the conditions leading to each protein secretion profile and, with bioinformatic analysis, to the identification of the gene expressing each protein. In this study, we identify the protein secretion profile from *B. cinerea* in response to strawberry receptacle tissues to demonstrate the power of the technology in elucidating changes in fungal secretion profiles upon exposure to plant tissue.**

543. Withdrawn

544. An ABC transporter in ***Nectria haematococca*** **MPVI is responsible for tolerance to pisatin and is involved in virulence on pea. Jeffrey J. Coleman and Hans D. VanEtten, Department of Plant Sciences, Division of Plant Pathology and Microbiology, University of Arizona, Tucson, AZ, 85721.**

The pea pathogen *Nectria haematococca* MPVI has evolved at least two tolerance mechanisms to the pea phytoalexin pisatin. The well characterized cytochrome P450 pisatin demethylase (PDA) catalyzes a one step demethylation of pisatin to form the less toxic (+)6a-hydroxymaackiain. The other tolerance mechanism is an inducible nondegradative mechanism believed to be the result of an ABC transporter. The genome of *N. haematococca* MPVI has a total of 78 of these transporters, in which five appear to be significantly up regulated upon challenge with pisatin. Two of these ABC transporters have been previously identified by RT-PCR from pisatin induced mycelia. The most expressed ABC transporter after pisatin challenge, *NhABC2*, is very similar (89% amino acid similarity) to the *Gibberella pulicaris* GpABC1 protein which is required for virulence on potato and tolerance to the potato phytoalexin rishitin. When *NhABC2* and *PDA* are disrupted in *N. haematococca* MPVI there is a greater reduction in virulence than either single mutant in pathogenicity assays and pisatin tolerance is significantly reduced. These results suggest that the two tolerance mechanisms are responsible for overcoming host phytoalexin production.

545. Cell wall remodeling and pathogenic development in *Magnaporthe grisea*. Rahim Mehrabi, Yangseon Kim, Shengli Ding, Xinhua Zhao, Jin-Rong Xu. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN, USA.

***Magnaporthe grisea* is an economically devastating phytopathogen causing the rice blast disease. We have previously characterized the *M. grisea MPS1* MAP kinase gene (the homolog of yeast *SLT2*) and found that appressoria formed by the *mps1* mutant failed to penetrate the plant cells. To further investigate the relation between cell wall integrity and pathogenic development, we examined the cell wall ultra-structures in the *mps1* mutant. Transmission electron microscopy (TEM) examination of appressoria showed that the appressorium cell wall of the *mps1* mutant is thinner, fussy, less melanized and often contains cracks that may cause inability of *mps1* appressoria to generate sufficient turgor pressure for mechanical penetration of the plant cells. TEM of the aerial hyphae showed that the *mps1* mutant lacks the capsule layer, has a thinner and un-even cell wall. Cell wall sugar analysis showed no significant difference in the total sugar content but the composition of cell wall monosaccharides were different between the WT and *mps1* mutant as detected by gas liquid chromatography. The *mps1* mutant has slightly more mannose and over 2x galactose whereas WT has more glucose. The *mps1* mutant showed hypersensitivity to several plant defense compounds including defensin, osmotin and hydrogen peroxide. Although *mps1* and WT strains had similar growth rates at 25ºC, the *mps1* mutant grew faster than the WT strain at lower temperatures, indicating that the *mps1* mutant had increased tolerance to cold stress. Two dimensional gel electrophoresis was used to identify proteins that are differentially expressed between the WT and the *mps1* mutant. Over 37 proteins have more than tree folds changes in their expression levels. We also characterized the MEK Mkk2 and the MEKK Bck1. Similar to the *mps1* mutant, the *bck1* and *mkk2* mutants were able to form appressoria but were unable to penetrate plant cells and failed to develop infectious hyphae, indicating that Bck1 and Mkk2 are upstream component of Mps1.**

546. Functional analyses of three G alpha and one G beta subunits in *Gibberella zeae.* Hye-Young Yu[1], Jeong-Ah Seo[1], Kap-Hoon Han[2], Sung-Hwan Yun[3] and Yin-Won Lee[1]. [1]School of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea [2]Department of Pharmaceutical Engineering, Woosuk University, Wanju 565-701, Korea [3]Department of Biological Resources and Technology, Soonchunhyang University, Asan 336-745, Korea

The homothallic ascomycetous fungus *Gibberella zeae* (anamorph: *Fusarium graminearum*) is a toxigenic plant pathogen that causes head blight disease on small grains and produces major mycotoxins, deoxynivalenol (DON) and zearalenone (ZEA). To better understand the nature of pathogenic process and toxin production, we identified and characterized heterotrimeric G-proteins that function in a crucial signaling pathway responsible for a variety of cell responses in fungi. Analysis of *G. zeae* genome identified three putative G alpha subunits, GzGPA1, GzGPA2 and GzGPA3, and one G beta subunit, GzGPB1. Deletion (D) of *GzGPA1* resulted in female-sterility and enhanced DON and ZEA production, indicating that GzGPA1 is required for normal sexual reproduction and repression of toxin biosynthesis. D*GzGPB1* mutant also produced high concentration of DON and ZEA, suggesting that both G alpha GzGPA1 and G beta GzGPB1 negatively control mycotoxin production. Deletion of *GzGPA2* caused restricted hyphal growth, reduced pathogenicity, and increased chitin accumulation on the cell wall, implying that GzGPA2 has multifunction in hyphal proliferation, pathogenic development, and cell wall composition. Similar to the D*GzGPA2* mutant, the D*GzGPB1* mutant exhibited reduced hyphal growth coupled with diminished pathogenicity. This study provides a basis for better understanding the complex signaling networks in controlling fundamental biological processes in the pathogenic and toxigenic *G. zeae*.

547. Transducin Beta-Like Gene 1 (*TBL1*) in *Magnaporthe grisea* is Essential for Host Locolization. Sheng-Li Ding, Jin-Rong Xu. Department of Botany and Plant Patholoty,Purdue University, West Lafayette, IN 47907

The *TBL1* gene was identified by random insertional mutagenesis in *Fusarium graminearum* to be required for wheat head infection. It is well conserved among Pyrenomycetes and may represent a novel fungal pathogenicity factor. To

further characterize its role in plant infection, we generated a *tbl1* deletion mutant in *Magnaporthe grisea*. The *tbl1* mutant was significantly reduced in conidiation and failed to cause typical blast lesions on rice seedlings. At 7 days post inoculation, the *tbl1* mutant had no defect in germination and appressorium formation. However, appressoria formed by the *tbl1* mutant were reduced in the efficiency of appressorial penetration. For those appressoria that were successful in penetration, the primary infectious hyphae had limited. These data indicate that *TBL1* is required for the extension of infectious hyphae and plant colonization in *M.grisea*. *TBL1* may be involved in overcoming plant defense response pre- and post-penetration.

548. Genetic analysis of hybrid histidine kinase genes of *Cryptococcus neoformans.* Li Hao-Man, Kiminori Shimizu, Akira Watanabe, Masashi Yamaguchi, Katsuhiko Kamei, Susumu Kawamoto. Research Center for Pathogenic Fungi, Chiba University, Japan. kshimizu@faculty.chiba-u.jp

Hybrid histidine kinases (HHKs) are known to regulate drug resistance, osomo- tolerance, pathogenicity and hyphal growth in fungal species. *Cryptococcus neoformans*, a human pathogen, possesses seven HHK genes in its genome. One of them, *CnNIK1*, has been functionally analyzed and its involvement in phenylpyroll resistance, pathogenicity and HOG1 MAP kinase regulation was shown. Here we analyzed functions of the remaining HHK genes, *CnHHK2*, *CnHHK3*, *CnHHK4*, *CnHHK5*, *CnHHK6* and *CnHHK7* by gene knockouts. *C. neoformans* B-4500 (mating type α, serotype D) derivative, a uracil requiring strain was used for genetic manipulation, and *CnHHK2*, *CnHHK5*, *CnHHK6* and *CnHHK7* genes were successfully replaced with a functional *URA5* gene. Resultant transformants were designated as TLHM2, TLHM3, TLHM1 and TLHM4, respectively. From TLHM3, TLHM1 and TLHM4, we could obtain strains with opposite mating type by mating with B-3502 (mating type a, serotype D), but not from TLHM2. Sexual mating between HHK (*CnHHK5*, *CnHHK6* and *CnHHK7*) deletion strains with opposite mating type loci could complete sexual development as that between wild type strains, suggesting that *CnHHK5*, *CnHHK6* or *CnHHK7* are not required for mating. Their involvement in pathogenicity will be also presented.

549. Functional characterization of the RxLR-EER translocation signal for delivery of oomycete effector proteins into host plant cells. Stephen C Whisson[1], Severine Grouffaud[1,2], Petra C Boevink[1], Anna O Avrova[1], Pieter van West[2], and Paul R J Birch[1]. [1] Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee, United Kingdom. [2] Aberdeen Oomycete Group, Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom. email swhiss@scri.ac.uk.

The oomycete *Phytophthora infestans* causes late blight, a re-emerging potato disease that precipitated the Irish famines in the nineteenth century. The recent cloning of the *Avr3a* avirulence gene from *P. infestans*, and additional avirulence genes from other oomycetes, highlighted the presence of peptide motifs RxLR and/or EER, hypothesized to be involved in transport of the AVR proteins into host plant cells. We have used the *Avr3a* gene to examine this motif in more detail. AVR3a is recognized inside host cells by the cytoplasmic R3a protein, and the RxLR-EER motifs are not required for this recognition. We generated *P. infestans* transformants with the RxLR or EER, or both motifs replaced by alanine residues. None of the alanine replacement AVR3a transformants were recognized by *R3a* plants. Native and alanine replacement variants of AVR3a were fused to the monomeric red fluorescent protein to localize secretion. AVR3a::mRFP was secreted only from the finger-like biotrophic haustoria. Alanine replacement AVR3a::mRFP transformants exhibited fluorescence at haustoria and also in the plant apoplast surrounding the haustoria, consistent with it not translocating into host cells but accumulating in and overspilling the extra-haustorial matrix. Progress towards further definition of the requirements for effector protein translocation by the RxLR-EER motifs will be presented.

550. Characterization of white collar 1 gene from Fusarium oxysporum encoding a putative photoreceptor. Carmen, Ruiz-Roldán[1], Victoriano, Garre[2] and M.I.G., Roncero[1]. [1]Departamento de Genética, Universidad de Córdoba, Córdoba, Spain. [2]Departamento de Microbiología y Genética, Universidad de Murcia, Murcia, Spain. ge2rurom@uco.es

Light regulates developmental and physiological processes in a wide range of organisms, including filamentous fungi. The *wc-1* and *wc-2* genes are the key elements involved in light signal transduction in *Neurospora crassa*, the best-studied system at the molecular level. Recently, the *cwc1* and *cwc2* genes from the human fungal pathogen *Cryptococcus neoformans*, orthologous to *N. crassa wc-1* and *wc-2* genes, have been shown to be involved in the virulence of this fungus. In our study, we have isolated and characterised the *fowc-1* gene of *F. oxysporum*, the orthologe of the blue light photoreceptor of *N. crassa*. Analysis of the deduced protein sequence reveals a putative activation domain, a putative conserved LOV domain, a putative PAS dimerization domain, a putative nuclear localization sequence and a Zn-finger DNA binding domain. Targeted disruption mutants for *fowc-1* gene were generated in order to determine its function in *F. oxysporum*. No differences in growth were observed between mutants and wild type strains, when grown on plates containing PDA or minimal medium, under different ligth conditions (constant white ligth, constant darkness, or 12 h light/12 h darkness photoperiods). However, knockout mutants were unable to produce aerial mycelium during growth on cellophane sheets on PDA plates under 12 h light/12 h darkness photoperiods, in contrast to the wild type strain.

Pathotypic behaviour of D*fowc-1* mutants was similar to wild type strain, either on tomato plants, on fruits or on apple slices, under different ligth conditions (constant darkness or 12 h light/12 h darkness photoperiods), indicating that this gene is not essential for pathogenicity of *F. oxysporum*. Determination of carotene pigments production by the mutants growing on PDA plates under 12 h light/12 h darkness photoperiods showed a decrease in the total amount of carotenes produced in comparison to the wild type. Nevertheless, mutants still respond to light, suggesting the implication of a second regulatory element on light activation of carotenogenesis. Studies of the expression of *fowc-2* gene and determination of sporulation rates by the mutants and the wild type under different ligth conditions are being carried out at present. Further characterization of the mutant strains will be presented.

551. Implications in pathogenesis of class V chitin synthases from *Fusarium oxysporum.* M. Martín-Urdíroz[1], C. Ruiz Roldán[1], J.A. González-Reyes[2] and M.I.G. Roncero[1]. [1]Dpto. Genética, Campus Rabanales C5 1[a], Universidad de Córdoba. 14071-Córdoba, Spain b72maurm@uco.es [2]Dpto. Biología Celular, Fisiología e Inmunología, Campus Rabanales C6 3[a], Universidad de Córdoba. 14071-Córdoba, Spain

***Fusarium oxysporum* f. sp. *lycopersici* is a soilborne pathogen that causes wilt disease in tomato plants. Fungal colonisation of plants brings cells of both organisms into close contact. Thus, it is conceivable that fungal cell wall molecules play an important role in the interactions with the hosts. Chitin, glucans, manans and glycoproteins are the main components of this fungal structure. Microscopy studies in the plant pathogen *Ustilago maydis* have demonstrated that class V chitin synthase, harbouring a N-terminal myosin motor domain, is crucial for hyphal development inside the plant tissues. We are studying two class V chitin synthase genes, named *chsV* and *chsVb*, with the aim of identifying the role of chitin in the interactions of *Fusarium oxysporum* with tomato plants. These genes have shown to be essential for virulence of *F. oxysporum* since single targeted disruption mutants on any of them are unable to infect tomato plants. The unsuccessful disease development might be due to the inability of adhesion or surface penetration into the root and subsequent colonisation of the plant vascular system. In order to identify the developmental stage blocked in the *Fusarium* defective mutants during the infection process, scanning electron microscopy images of *F. oxysporum* wild type and D*chsV* strains growing on tomato roots have been analysed. In contrast to the wild type strain, germlings of D*chsV* mutant did not penetrate but formed large aggregates around the tomato roots. Transmission electron microscopy analysis of plants inoculated with spores from D*chsV* and D*chsVb Fusarium* mutants are being currently performed to investigate hyphal growth from D*chsV* or D*chsVb* mutants inside host tissues.**

552. Functional Analysis of the *Phytophthora sojae* effector protein Avr1b. Daolong Dou [1], Rays H.Y. Jiang [1,2], Xia Wang [1], Shiv Kale [1], Felipe Arredondo [1], Sucheta Tripathy[1] and Brett M. Tyler [1]. [1]Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA24061. [2] Laboratory of Phytopathology, Wageningen University, NL-6709 PD Wageningen, The Netherlands and Broad Institute, Cambridge, MA02141.

The genome sequences of several oomycete plant pathogens, including the soybean pathogen *Phytophthora sojae*, reveal that each of these genomes encodes several hundred proteins with sequence similarity to cloned oomycete avirulence genes. We have identified conserved motifs present in large numbers of these genes, including the *P. sojae* avirulence gene *Avr1b* -1. We have tested the function of the motifs in *Avr1b* -1 using *P. sojae* stable transformants and soybean transient expression assays. The RXLR motif, found near the N terminus of most of the effectors, is required for *Avr1b* -1 to confer avirulence when expressed in *P. sojae*, but not when expressed inside soybean cells, supporting the hypothesis that RXLR is required for Avr1b proteins to transit into plant cells. The sequences surrounding the RXLR motif are also required, but there is only a weak requirement for the adjacent dEER motif. Avr1b contains two C terminal motifs that occur as a pair in approximately half of all the *P. sojae* effectors, and both are required to confer avirulence. Overexpression of *Avr1b* -1 increases the virulence of *P. sojae* transformants on susceptible cultivars and we are currently determined the role of the conserved motifs in conferring increased virulence.

553. Chitosan, the Deacetylated Form of Chitin, is Necessary for Cell Wall Integrity in *Cryptococcus neoformans.* Baker, Lorina G.[1], Specht, Charles A.[3],Donlin, Maureen J.[1,2],and Lodge, Jennifer K.[1,2]. Edward A. Doisy Department of Biochemistry and Molecular Biology[1] and Department of Molecular Microbiology and Immunology[2], Saint Louis University School of Medicine, 1402 S. Grand Boulevard, Saint Louis, Missouri 63104, and Department of Medicine[3], University of Massachusetts, 364 Plantation Street, Worcester, Massachusetts 01605

***Cryptococcus neoformans* is an opportunistic fungal pathogen that causes cryptococcal meningoencephalitis, particularly in immunocompromised patients. The fungal cell wall is an excellent target for anti-fungal therapies as it is an essential organelle that provides cell structure and integrity, is needed for localization or attachment of known virulence factors, including the polysaccharide capsule, melanin and phospholipase, and is critical for host-pathogen interactions. In *C. neoformans*, chitosan produced by the enzymatic removal of acetyl groups from nascent chitin polymers has been implicated as an important component of the vegetative cell wall. In this study, we identify four putative chitin/polysaccharide deacetylases in C. neoformans. The substrate for one, Fpd1, is undetermined at present, but we**

have demonstrated that the other three, Cda1, Cda2, and Cda3, can account for all of the chitosan produced during vegetative growth in culture. The data suggests a model for chitosan production in vegetatively growing *C. neoformans* where the three chitin deacetylases convert chitin generated by the chitin synthase, Chs3, into chitosan. Utilizing a collection of chitin/polysaccharide deacetylase deletion strains, we determined that during vegetative growth, chitosan helps to maintain cell integrity and aids in bud separation. Additionally, chitosan is necessary for maintaining normal capsule width, and lack of chitosan results in a "leaky melanin" phenotype. Our analysis indicates that chitin deacetylases and the chitosan made by them may prove to be excellent anti-fungal targets.

554. Withdrawn

555. Identification of *Fusarium oxysporum* cell surface proteins regulated by the Pathogenicity MAP kinase cascade using 2D-DIGE. Rafael C. Prados-Rosales[1], Concha Gil[2], and Antonio Di Pietro[1]. [1]Departamento de Genética, Universidad de Córdoba, Spain [2]Departamento de Microbiología II, Universidad Complutense de Madrid, Spain

***Fusarium oxysporum* is a soilborne pathogen that causes vascular wilt disease in a large number of plant species. During the initial stages of infection, the fungus attaches to the roots and initiates hyphal growth on the root surface. We have previously found that the MAP kinase Fmk1 is essential for root attachment and pathogenicity of *F. oxysporum*. We hypothesized that fungal adhesion to roots could be mediated by cell wall proteins (CWPs) present at the hyphal surface. A proteomic approach was used to compare CWPs present in the wild type strain and the *fmk1* mutant. Total CWPs were extracted from spores germinated under adhesion-inducing conditions and fractionated according to their interaction with structural cell wall components. Four fractions were obtained by consecutive treatments with hot SDS, mild alkaline, Quantazyme and exochitinase. The enriched CWP fractions from the wild type and *fmk1* mutant were compared by two-dimensional differential in-gel electrophoresis (2D-DIGE). A number of differentially expressed proteins have been identified in each fraction and are being characterized by mass spectrometry. The results of this study should provide novel insights into the role of the Pathogenicity MAP kinase cascade in shaping the fungal cell surface.**

556. Discovery of secretome signals in interaction between insect host and Zygomycete pathogens. Lene Lange*, Annette Bruun Jensen', Morten Nedergaard Grell', Eva Holm Hansen*, Peter Bjarke Olsen*, and Jørgen Eilenberg'. *Novozymes, Molecular Biotechnology, 2880 Bagsværd, Denmark. 'Agricultural University, Department of Ecology, Thorvaldsensvej 40, Denmark. lla@novozymes.com

Screening of cDNA libraries made from fungal infected cereal aphids (*Sitobion avena*, infected with *Entomophthora planchoniana, Pandora neoaphidis, Conidiobolus obscurus, Entomophthorales, Zygomycetes*), specifically targeting the secretome of interacting organisms, has led to a series of interesting discoveries, including secreted enzymes and pathogenesis related proteins. Such interaction studies allow for gene discoveries of obligate biotrophic organisms where only very limited amino acid and base pair sequence information is available. The targeted secretome studies were made possible through application of the transposon assisted signal trapping (TAST, Becker et al., J.Microbial Methods, 2004, 57(1), 123-33) for screening of the cDNA library. The rich results is further related to that the total RNA (used as basis for isolating mRNA and subsequent for constructing the cDNA library) was made from directly harvesting of early infection stage of aphids (*S. avenae*), attacked by Entomophthoralean pathogens, spotted and collected in the field in an early summer morning in Denmark, 2005.

557. Withdrawn

558. The mitochondrial genome of Fusarium species encode an exceptionally large, novel open reading frame. Anthony Herring, SiZhi Zhou, Akhil Pai, Rahul Malireddy and John C. Kennell. Department of Biology, Saint Louis University, St. Louis, MO 63101-2010

The mitochondrial (mt) genomes of F. graminearum, F. solani and F. verticilliodes have been sequenced and annotated. The mtDNAs are 95,676 bp, 62, 979 bp and approximately 54,000 bp, respectively. All three genomes encode genes typically associated with fungal mitochondria and the order of genes encoding polypeptides is syntenic with mtDNAs of Fusarium oxysporum. The three-fold size difference in Fusaria mt genomes is due largely to an increased number of group I introns and non-coding regions. Polypeptide encoding regions are syntenic, yet there are differences in the number and location of tRNAs. Surprisingly, the genomes of F. graminearum, F. solani and F. verticilliodes contain an exceptionally large unidentified open reading frame (ORF) predicted to encode polypeptides of 1931, 2013, and 2572 amino acids in length. The predicted polypeptides have several transmembrane domains, suggesting they may function in the inner mitochondrial membrane. The G+C content and codon usage of the large ORFs indicate that they have not co-evolved with other mt genes and are recently acquired. An additional small unidentified ORF was detected in all four Fusarium species and ratio of nonsynonymous to synonymous mutations indicates that the ORF is under stabilizing

selection. If functional, the acquisition of these new genes contrasts to the wide-scale size reduction experienced by fungal mitochondrial genomes and may reveal functions that are unique to Fusarium species.

559. Centromere assembly and maintenance in *Neurospora crassa.* Lanelle R. Connolly, Grant Farr, Joseph Mendoza and Michael Freitag. Dept. of Biochemistry and Biophysics, Center for Genome Research and Biocomputing, Oregon State University, USA

Centromeres form the platform for the assembly of kinetochores, the attachment points for spindle microtubules that transport chromosomes into daughter nuclei during nuclear division. Precisely what constitutes functional centromeres remains unclear in most eukaryotes. In addition to a specific histone H3 variant, CenH3, typical centromeres are associated with repeated DNA. Centromeric nucleosomes also carry specific posttranslational histone modifications. One of our goals is to assess the contribution of repeated DNA and histone modifications to the placement of functional centromeres in filamentous fungi. To identify proteins involved in centromere and kinetochore assembly and maintenance, we have generated fusions of centromere-specific genes (CenH3, Cenp-I and Cenp-S) with fluorescent proteins and affinity tags. We will report on the identification of centromere-associated DNA of *Neurospora crassa*, the characterization of genes for centromeric histone variants from filamentous fungi, and the identification of proteins involved in centromere assembly.

560. Evidence for Biofilm Formation by Pneumocystis. Melanie T. Cushion and Margaret S. Collins. University of Cincinnati College of Medicine, Cincinnati, OH 45267-0560 and the Cincinnati VAMC, Cincinnati, OH 45220

Organisms in the fungal genus Pneumocystis, cause an oftentimes lethal pneumonia (PCP) in mammals with compromised immune status. Limited progress has been made in understanding the life cycle, transmission, and natural history of Pneumocystis species, due in large part to the absence of a continuous in vitro culture system. Development of efficacious therapeutics has been especially hindered. Recent reports suggest the presence of P. jirovecii in patients with underlying chronic diseases like COPD, may serve as a co-morbidity factor. Molecular and serological studies in animals and humans suggest Pneumocystis is also present in the lungs of non-immunocompromised hosts. The strategies used by these organisms to grow and survive in the context of an intact or debilitated host defenses are largely unknown. During infection, the Pneumocystis attach specifically to Type I pneumocytes and produce large packed clusters that eventually spread throughout the alveolus, fill the lumen, and expand throughout the lung. We posit that this process is similar to biofilm formation and this structure affords the same advantages as it does to other fungi that form biofilms, specifically, resistance to antimicrobial therapy, a mode for dissemination of infection, and resistance to host immune clearance. To study the process, an in vitro system was identified that supported biofilm formation by *P. carinii* (from rat) and *P. murina* (from mouse). Biofilms were established on insert wells placed in multi-well plates or on treated chamber slides after 3 days in a supplemented RPMI 1640 medium and maintained for up to 3 weeks at 36C in reduced oxygen atmospheres. Specific morphological changes accompanied the biofilm formation including an increase of refractility of organisms clusters associated with an exclusion of tinctorial staining; establishment of microclusters; and a white film that increased in size and mass over time. Confocal microscopy with FUN-1 and ConA-AlexaFluor showed the organisms were viable and enmeshed in an exopolymeric matrix within a 3-dimensional structure. Biofilm formation was quantified by an ATP-driven bioluminescent assay which showed a decrease in ATP of planktonic (non-adherent) cells and an increase in sessile (biofilm cells). Quantitative Reverse Transcriptase PCR directed to the dihydrofolate reductase gene of *P. carinii* from the sessile cells was relatively stable after initiation of the biofilm, indicating a slow down in metabolism, such as that observed in other fungal biofilms. The biofilms could be passaged to naïve inserts and inoculation of these secondary biofilms were able to cause infection in immunosuppressed rats, supporting the viability and infectivity of these cells. Signficantly, the biofilms had reduced susceptibility to TMP-SMX and grew under increased CO2 levels. These collective data support our contention that Pneumocystis species form biofilms, which we posit is a survival strategy used by this genus. Optimization of the in vitro system will fundamentally advance the field of Pneumocystis.

561. Determining the role of reactive oxygen species generation in *Magnaporthe grisea*. Elise Lambeth, Martin J. Egan and Nicholas J Talbot. School of Biosciences, University of Exeter, Geoffrey Pope Building, Perry Road, Exeter EX4 4QD, United Kingdom

Recent studies have demonstrated that reactive oxygen species actively participate in a diverse array of biological processes, including normal vegetative cellular growth, induction and maintenance of the transformed state in mammalian cells, programmed cell death and cellular senescence. NADPH oxidases (Nox) are flavoenzymes used to generate reactive oxygen species (ROS). Until recently, research into Nox and ROS generation was focussed primarily on the oxidative burst associated with the plant defence response and leukocyte function. However, the recent discovery of new functional members of the Nox family has implicated ROS in many diverse processes including growth and sexual development in a wide variety of organisms including filamentous fungi. Here, we investigate the role of NADPH oxidase-generated ROS in the infection-related development of the phytopathogenic ascomycete fungus *Magnaporthe grisea*. This

fungus 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 an appressorium allows the fungus to breach the host cuticle using mechanical force and subsequently cause plant infection. *M. grisea* possesses three NADPH oxidase homologues. We have shown that delta nox1 mutants exhibit abnormal germ tube morphology and fail to cause rice blast disease. delta nox2 mutants also fail to cause rice blast disease symptoms due to a defect in appressorium-mediated penetration. The current project concentrates on characterising and localising the NOX3 gene product, which is the third member of the family of *M. grisea* NADPH oxidases and also to identify a possible regulator of NADPH oxidases.

562. Comparison of gene inventories of phytopathogenic and saprotrophic filamentous ascomycetes. Darren M. Soanes, Han Min Wong and Nicholas J. Talbot. School of Biosciences, University of Exeter, Geoffrey Pope Building, Perry Road, Exeter EX4 4QD, United Kingdom

As the number of completed fungal genome sequences increases, there is a need for resources and tools to integrate and analyse this data. e-Fungi aims to integrate sequence and functional data from multiple fungal sequences, facilitating the systematic study of less well understood species with reference to model organisms. The e-Fungi data warehouse currently contains genomic sequence data from thirty-six fungal species, including model organisms, non-pathogenic fungi and both human and plant pathogens. This is integrated with functional data from a variety of sources. e-Fungi consists of a data warehouse and a library of bioinformatics queries and analyses, which can be combined in different ways to conduct studies of cellular processes, pathogenicity and evolution. Both warehouse and analysis libraries are available within a service- oriented GRID. Molecular phylogeny has shown that pathogenic fungi are found in many taxonomic groups, which suggests that these lifestyles have evolved repeatedly within the fungal kingdom. Comparison of genomic and functional data between pathogenic and non-pathogenic species of fungi will enable us to start to answer the question "What makes a pathogen different from a non-pathogen?" We have compared the gene inventories of five species of phytopathogen (*Magnaporthe grisea, Botrytis cinerea, Gibberella zeae, Septoria nodorum* and *Sclerotinia sclerotiorum*) to four species of saprophyte (*Aspergillus nidulans, Chaetomium globosum, Neurospora crassa* and *Trichoderma reesei*). We have identified Pfam motifs that are more common in the genomes of the phytopathogens than saprotrophs. This may be due to gene family expansion and include genes putatively involved in secondary metabolism, plant cell wall degradation, stress response and signal transduction. MCL clustering of protein sequences has enabled us to identify further cases of pathogen-specific genes. We have also characterised their predicted secretomes and classified these according to predicted function. Progress in the establishment and further development of the e-Fungi data warehouse and the COGEME Phytopathogen EST database (http://cogeme.ex.ac.uk) will be presented.

563. The *Penicillium expansum* Gox2 Gene Is Required for Virulence on apple fruits. Oren Moskovitch, Yoav Hadas, Amnon Lichter and Dov Prusky. Department of Postharvest Science of Fresh produce, Agricultural Research Organization, Bet Dagan 50250. dovprusk@agri.gov.il.

Filamentous fungi can alter their environmental pH during attack of plant tissue, presumably in order to modulate fungal attack. Gluconic acid is the major metabolite secreted by *Penicillium expansum* that can alter ambient pH. Gluconic acid is catalyzed by oxidation of glucose by glucose oxidase and it hypothesized that this process may be very significant to the virulence of P. exapansum on the glucose reach apple fruit after harvest. Under conditions of reduced O2 (10 Kpa), the virulence of *P. expansum* is reduced by 15%. Expression of Gox2, one of the two glucose oxidase genes identified in *P. expansum*, was reduced under these conditions. Loss-of function gox2 mutants were dramatically impaired in gluconic acid production and virulence on apple fruit. Supplementing the mutants with gluconic acid restored pathogenicity symptoms. Based on these results, Gox2 appears to be necessary for the pathogenesis of *P. expansum* on apple fruit after harvest

564. Transcrptional response to terpenoids, antimicrobial defense compounds of lodgepole pine, in the sapstain fungus *Ophiostoma clavigerum*. Scott DiGuistini[1], Jörg Bohlmann[2] and Colette Breuil[1]. [1]Department of Wood Sciences, University of British Columbia, Vancouver, BC, Canada. [2]Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada

***Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington, is a lodgepole pine pathogen associated with the bark beetle, *Dendroctonus ponderosae* (Mountain Pine Beetle, MPB). Lodgepole pine defenses against the bark beetle and fungus include the biosynthesis of terpenoids and phenolics, some of which may be deterrent or toxic to MPB and MPB-associated fungi. Within a few weeks of bark beetle attack fungal growth can occupy the entire sapwood region. It is difficult to separate the destructive nature of MPB and staining fungi, due to their close symbiotic relationship. However, bark beetle-vectored blue stain fungi contribute significantly to tree mortality. To examine molecular processes associated with the early stages of *O. clavigerum* host colonization, we are exposing cultured *O. clavigerum* to a select set of pine oleoresin terpenoids followed by gene expression profiling over a three-day period. In this work we will present and discuss a comparison between two transcriptome profiles of *O. clavigerum* generated 12 hrs. after treatment with and**

without terpenes, as well as quantitative real-time PCR data in support of this profiling. An understanding of metabolite induced fungal gene expression may provide clues as to the mechanisms of antimicrobial metabolite activity and fungal tolerance towards these compounds.

565. Phylogenetic diversity and SNP-based genotyping of fusaria associated with the 2005-06 multistate keratitis outbreaks among soft contact lens wearers within the U.S. Kerry O'Donnell[1], Brice Sarver[1, 2], Mary Brandt[3], Douglas Chang[3], Judith Nobel-Wang[3], Carol Rao[3], Scott Fridkin[3], Benjamin Park[3], Arvind Padhye[3], Deanna Sutton[4], David Geiser[5], and Todd Ward[1]. [1]NCAUR, Peoria, IL. [2]Washington University in St. Louis, St. Louis, MO. [3]CDC, Atlanta, GA. [4]University of Texas, San Antonio, TX. [5]Pennsylvania State University, State College, TX.

In 2005-06, outbreaks of Fusarium keratitis occurred in multiple U.S. states and Puerto Rico associated with soft contact lens use. A case-control study conducted by the Centers for Disease Control and Prevention (CDC) showed a significant association between infections and the use of one particular brand of lens solution. To determine the cause and potential sources of the pathogen, partial DNA sequences from 3 loci (RPB2, EF-1α, and nuclear ribosomal rRNA) totaling 3.48 kb were obtained from 87 corneal cultures and 104 isolates from the patient's environment (e.g., contact lens and lens cases). We also sequenced a 1.8 kb region encoding the second largest RNA polymerase (RPB2) subunit from 126 additional pathogenic isolates, to better understand how the keratitis outbreak isolates fit within the full phylogenetic spectrum of clinically important fusaria. Seventy-two of the aligned RPB2 sequences, chosen to represent the phyletic diversity of Fusarium, were analyzed by maximum parsimony to develop a global phylogenetic framework. In addition, RPB2 nucleotide variation within the 72 isolate panel was used to design 34 allele-specific probes to identify representatives of all 6 medically important species complexes and 10 of the most important human pathogenic Fusarium species in a single-well assay, using flow cytometry and fluorescent microsphere technology. The multilocus genotyping data show that one haplotype of the 3 most common species comprised over 50% of CDC's corneal and environmental isolates, and that the corneal cultures comprised 33 haplotypes distributed among 17 species. The high degree of phylogenetic diversity represented among the corneal isolates is consistent with multiple sources of contamination.

Model Systems

566. Molecular Mechanism of Interlocked Feedback Loops in *Neurospora* Circadian Clock. Qun He, Joonseok Cha, Qiyang He, Heng-Chi Lee, Yuhong Yang, and Yi Liu. Department of Physiology, The University of Texas Southwestern Medical Center, Dallas, Texas 75390.

The eukaryotic circadian oscillators consist of circadian negative feedback loops. In *Neurospora*, it was proposed that the FREQUENCY (FRQ) protein promotes the phosphorylation of the WHITE COLLAR (WC) complex, thus inhibiting its activity. The kinase(s) involved in this process is not known. In this study, we show that the disruption of the interaction between FRQ and CK-1a (a casein kinase I homolog) results in the hypophosphorylation of FRQ, WC-1, and WC-2. In the *ck-1a*^{L} strain, a knock-in mutant that carries a mutation equivalent to that of the Drosophila *dbt*^{L} mutation, FRQ, WC-1, and WC-2 are hypophosphorylated. The mutant also exhibits ¡-32 h circadian rhythms due to the increase of FRQ stability and the significant delay of FRQ progressive phosphorylation. In addition, the levels of WC-1 and WC-2 are low in the *ck-1a*^{L} strain, indicating that CK-1a is also important for the circadian positive feedback loops. In spite of its low accumulation in the *ck- 1a*^{L} strain, the hypophosphorylated WCC efficiently binds to the C-box within the *frq* promoter, presumably because it cannot be inactivated through FRQ-mediated phosphorylation. Furthermore, WC-1 and WC-2 are also hypophosphorylated in the *cka*^{RIP} strain, which carries the disruption of the catalytic subunit of casein kinase II. In the *cka*^{RIP} strain, WCC binding to the C-box is constantly high and cannot be inhibited by FRQ despite high FRQ levels, resulting in high levels of *frq* RNA. Together, these results suggest that CKI and CKII, in addition to being the FRQ kinases, mediate the FRQ-dependent phosphorylation of WCs, which inhibit their activity and close the circadian negative feedback loop.

567. Response of *Neurospora crassa* strains expressing archaeal genes superoxide reductase and rubrerythrin reductase to the fungal toxin cercosporin. Sonia Herrero[1], Yang Ju Im[1], Amy Grunden[2], Wendy Boss[1] and Margaret Daub[1]. [1] Department of Plant Biology. North Carolina State University. Raleigh, NC 27695. [2] Department of Microbiology. North Carolina State University. Raleigh, NC 27695.

Cercosporin is a virulence factor produced by several members of the plant pathogenic genus *Cercospora*. In the presence of cercosporin and light, oxidative stress is induced in host plants due to the formation of reactive oxygen species (ROS) such as singlet oxygen and superoxide. Thus, control strategies to reduce the damage cause by these fungi and their toxin must be targeted at reducing production of ROS. The genetic mechanisms underlying resistance to cercosporin in

***Cercospora* spp. have proved elusive, however we know that transient reduction of cercosporin in the fungus leads to lower singlet oxygen production. To further our understanding and to identify genes that may provide resistance to cercosporin, we began investigating if genes from heterologous organisms known to be involved in resistance to oxidative stresses provide protection against cercosporin toxicity. To this effect, we have selected two candidate genes from the hyperthermophilic archaeon *Pyrococcus furiosus*: superoxide reductase (SOR) and rubrerythrin reductase (Rr). These genes are involved in the detoxification of superoxide and hydrogen peroxide, respectively, and provide protection against different oxidative stresses when expressed in *Arabidopsis thaliana*. We have transformed the cercosporin-sensitive fungal model *Neurospora crassa* with SOR and Rr and are presently testing transformed strains for their response to cercosporin and additional oxidative stresses induced by heat, hydrogen peroxide and multiple generators of superoxide radicals.**

568. A putative MAP-kinase, MpkB, regulates natural product biosynthesis in *Aspergillus nidulans.* Dapeng Bao and Ana M. Calvo. Department of Biological Sciences, Northern Illinois University, 1425 W Lincoln Hwy., Dekalb, Illinois. 60115, USA

Mitogen-activated protein kinase pathways transduce a large variety of external signals. This essential funtion is conserved in many eukaryotes, including fungi. In this work we studied the role of *mpkB*, encoding a putative homolog of the *Saccharomyces cerevisiae* FUS3 MAP-kinase, on the biosynthesis of natural products in the model filamentous fungus *Aspergillus nidulans*. We found that the *mpkB* loss-of-function mutant not only presented reduced production of the mycotoxin sterigmatocystin but also showed alterations in the biosynthesis of other natural products. The *mpkB* mutant only produced trace amounts of penicillin under conditions that promoted the production of this antibiotic in the wild type (approximately 27-fold greater). Furthermore, we found that expression of *ipnA*, encoding an isopenicillin synthase, is substantially reduced in the *mpkB* mutant. The complemented strain restored sterigmatocystin and penicillin wild-type levels. These results suggest that the MAPK signaling cascade regulates secondary metabolism in an adaptive response to environmental stimuli.

569. Withdrawn

570. Withdrawn

Other subjects

571. Identification of Two Novel Proteins as a Developmental Regulator Le.cdc5 Partner in the Basidiomycetous Mushroom *Lentinula edodes*. Kazuo Shishido, Takehito Nakazawa, and Shinya Kaneko Dept. of Life Science, Grad. Sch. of Biosci. and Biotechnol., Tokyo Institute of Technology, Yokohama, Japan. e-mail: kshishid@bio.titech.ac.jp

***Lentinula edodes* Le.CDC5 (842 aa) is a c-Myb-type transcription factor that contains a leucine zipper between two A kinase-sites (in C-terminus), nuclear localization signals, proline-rich and acidic aa-rich domains. It plays roles in morphological differentiation in all stages after fruiting initiation of L . edodes . We identified two proteins interacting with Le.CDC5, which were named CIPA and CIPB (Le. C DC5- I nteraction P artner A or B). CIPA (320 aa) contained a leucine zipper (in C-terminus), proline-rich, acidic and hydrophobic aa-rich regions and an A kinase-site. Almost all (302) aa sequences of CIPA were shown to be partially homologous to the sequences in the Dictyostelium homeobox transcription factor HBX2. CIPB (127 aa) contained a leucine zipper (in C-terminus) and proline-rich sequence and it was considered to be a homologue of the hypothetical proteins in other basidiomycete fungi. The A kinase-catalyzed phosphorylation of Le.CDC5 prohibited the interaction with CIPA and CIPB. It was shown that cipA gene is abundantly transcribed in primordia and mature fruiting bodies, whereas the cipB is transcribed at similar levels during fruiting-body formation. The cipA transcript was present in outer region of trama and subhymenium, whereas the cipB transcript in hymenium.**

572. Desperately seeking Kingdom Fungi. David Moore, Faculty of Life Sciences, The University of Manchester, UK, david.moore@manchester.ac.uk

The one place it's difficult to find fungi is in the school curriculum. Although we're surrounded by, and dependent on, fungi every hour of every day of our lives, the school curriculum doesn't seem to know they exist. In the UK, the word 'fungus' does not appear in the 87-page Programme of Study for Science, which is the statutory instrument that defines the curriculum for education from ages 5 to 16. And this seems to be a world-wide problem (the search engine on the NSES website at http://newton.nap.edu/html/nses/ finds only one line containing the string 'fung*' in the text of the National Science Education Standards). Few children anywhere on Earth are told about Kingdom Fungi as a third eukaryote kingdom given equal rank alongside animals and plants. Over the past few years, the British Mycological Society has produced an ever-widening range of teaching resources that are made freely available to schools to help

combat this educational deficiency. Our belief is that if young children are not taught properly about the place of fungi in world biology then older children and teenagers will not know enough about them to choose to study fungi further at college and university level. These resources will be demonstrated (and distributed).

573. Utilizing *Coprinus cinereus* to demonstrate mutagenesis and genetic screening in an undergraduate laboratory exercise. Marilee A. Ramesh and Nicole Randall. Department of Biology, Roanoke College, Salem, VA., ramesh@roanoke.edu

The basidiomycete *Coprinus cinereus* was utilized as a model to demonstrate the concepts of mutagenesis and genetic screens in an upper level undergraduate laboratory exercise. In this laboratory, students conducted a UV mutagenesis of *C. cinereus* asexual spores and screened the surviving isolates for meiotic defects. *C. cinereus* was selected because of its natural meiotic synchrony, making it an excellent model system to study meiosis. Meiotic mutants can be easily identified on the basis of reduced spore production. The mutagenesis was performed using a self-compatible strain, AmutBmut, to facilitate an efficient screen by eliminating the need to perform multiple crosses before the spore phenotype could be scored. The survivors of the mutagenesis were allowed to fruit and were scored for the ability to produce spores. This laboratory exercise served to illustrate a difficult concept for students, namely, how a genetic screen can be used to separate one class of mutations from a diverse group of genetic defects caused by random DNA damage. This laboratory provided students with hands-on experience in conducting a mutagenesis, evaluating the % kill through viability tests, collecting survivors and screening survivors for spore-producing abilities. The experience provided the student with both technical skills and the opportunity to evaluate the outcomes of the laboratory exercise.

574. Ergot alkaloid synthesis gene clusters in *Claviceps fusiformis, Neotyphodium coenophialum* and *Epichloë festucae*. E. Konnova[1], C. Machado[1], M. Liu[2], L. Gill[1], D. G. Panaccione[3] and C. L. Schardl[1].

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Clusters of ergot alkaloids synthesis (*EAS*) genes in *C. fusiformis, N. coenophialum* and *E. festucae* were cloned, sequenced and analyzed. The cluster in *C. fusiformis* (SD58= ATCC26245, cosmid 26E11) is located on a 19.6 kb genomic region and includes 9 genes. Comparative analysis of this cluster with the one from *C. purpurea* (P1=ATCC20102), which is composed of 13 genes, showed that the middle portion of the cluster is rather conserved between the two fungi. But, gene disruption and rearrangement was observed at the left border of the *C. fusiformis* cluster where positions of genes encoding *Cf-lpsB* and *Cf-easA* differ from those in *C. purpurea*. Moreover, numerous other changes have inactivated *Cf-lpsB*. To date, two *EAS* gene clusters have been identified in *N. coenophialum* isolate e19; one (cosmid 28B11) has *dmaW* linked to *easH* and genes for two more hypothetical proteins. In contrast, the *EAS* cluster of *C. purpurea* has no hypothetical protein genes between the above cluster genes. In another fosmid (136p13) from *N. coenophialum*, *easC* and *easD* were linked to *lpsA*. The *E. festucae* (isolate E2368) clusters were identified from the fungal genome sequence. To date, 11 genes for enzymes of the EAS pathway have been located in 5 contigs of the *E. festucae* genome sequence, and clustering of *dmaW*, *cloA*, *easC* and *easD* was confirmed by sequencing fosmids 7D14 and 40M3 from this isolate. Some microsynteny characterizes *E. festucae, C. purpurea* and *C. fusiformis EAS* clusters, whereas *N. coenophialum EAS* genes located to date show rather distinct gene order and orientation. Thus, the *Claviceps* species showed greater conservation of EAS cluster synteny than did the endophytes.

575. Some oddities of Fusarium transposable elements: gain of introns in pogo-like elements and cut-only transposition mechanism for a Mutator–like element. Marie Dufresne, Aurélie Hua-Van, Jean-Michel Davière, Christelle Vasnier, Laure Teysset and Marie-Josée Daboussi. Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France

Pogo-like elements, first recognized in animals and in fungi, are DNA transposons related to the Tc1/mariner super-family. They are widespread in filamentous fungi. Phylogenetic analysis is consistent with a diversification of multiple lineages, which can coexist in the same fungal species. Interestingly the large majority of pogo-like elements do not contain introns but in four transposase genes, introns were detected. There is no conservation in sequences among species or in the position. Each type of intron is restricted to a particular lineage. This patchy distribution can be explained more parsimoniously by the independent addition of introns as observed for MLEs in plants. Mutator-like elements are also present in some fungal species. The Hop element was the first active member identified outside the plants. Using a phenotypic assay for excision, we demonstrated that Hop is able to transpose in heterologous species. Hop can excise at high frequency but it does not seem to leave a footprint at the excision site and to reinsert in the genome. However, using a double selection procedure on very large cell populations, we found rare reinsertion events. These unusual properties are discussed in light of the structure and gene organization of Mutator-like elements

576. Recombinant expression of the Penicillium chrysogenum antifungal protein PAF in *Pichia pastoris* for structure-function analysis. Florentine Marx, Andrea Eigentler, Renate Weiler-Görz, Ulrike Binder. Biocenter, Division of Molecular Biology, Innsbruck Medical University, Innsbruck, Austria e-mail: florentine.marx@i-med.ac.at

We are currently investigating the impact of specific amino-acid residues of the small, cystein-rich and cationic extracellular protein PAF of *Penicillium chrysogenum* on its tertiary structure and its antifungal toxicity. To this end, the generation of large amounts of recombinant mutated PAF protein forms need to be generated. Aggravating for this project is the fact that PAF contains a prosequence between the signal sequence and the mature protein which plays an important role in protein folding and which is removed upon secretion of the protein. In our previous attempts, reasonable amounts of recombinant PAF were produced in a prokaryotic expression system, but the purified protein was inactive due to incorrect protein folding. Heterologous expression in *A. nidulans* as well as expression in *P. chrysogenum* resulted in the generation of active recombinant protein, but protein recovery was low. With the *Pichia pastoris* expression system we were able to produce high amounts of secreted recombinant PAF from paf cDNA that has been cloned into the multicopy expression vector pPIC9K. The secretion of the recombinant protein is driven by the alpha-factor secretion signal of *S. cerevisiae.* Toxicity assays revealed an antifungal activity comparable to that of the native protein. N-terminal protein sequencing and mass-spectroscopic analysis are currently employed to confirm the identity of the recombinant protein. The study of the structure-function relation of PAF will provide us with important information to develop new and efficient antifungal treatments in medicine, in agriculture and in the food industry. This work has been supported by the Austrian National Bank and by the Austrian Science Foundation FWF to FM.

577. Immunological characterization of an abundant cell wall antigen associated with saprotrophic growth and sporulation of the pathogenic and allergenic fungus Aspergillus fumigatus in soil. Christopher R. Thornton. School of Biosciences, University of Exeter, Washington Singer Labs., Perry Road, Exeter, UK, EX4 4QG. e-mail: C.R.Thornton@ex.ac.uk

***Aspergillus fumigatus* is a common saprotrophic fungus that is an opportunistic pathogen of humans. Despite its importance as a pathogen and allergen, very little is known about its basic biology. The natural habitat of *A. fumigatus* is soil, which represents the principal reservoir of propagules and associated antigens of the fungus. This work describes the development of a monoclonal antibody (JF5) raised against an abundant cell wall antigen. MAb JF5 reacts with antigens from Aspergillus species and species in the genera Emericella, Eurotium and Neosartorya. It also reacts with certain Penicillium spp., but does not recognize a wide range of unrelated fungi including the pathogenic and allergenic fungi *Cladosporium herbarum* and *Stachybotrys chartarum.* The antibody was used to monitor antigen production by *E. nidulans* and *A. fumigatus* during saprotrophic growth and sporulation in soil. Studies with *E. nidulans* A4 and the non-sporulating mutant brlA, showed that antigen production was associated with saprotrophic activity, in addition to sporulation, and confirmed immunofluorescence and immunoelectron microscopy observations that the antigen was secreted from the apex of active hyphae. The N-terminal of the antigen shows strong homology to PhiA. Gene deletion is currently being used to determine the effect of antigen disruption on saprotrophic competence of *A. fumigatus* in soil.**

578. Comparison of protein coding gene contents of fungal phyla *Pezizomycotina* and *Saccharomycotina.* Mikko Arvas[1], Teemu Kivioja[2], Alex Mitchell[3], Markku Saloheimo[1], David Ussery[4], Steve Oliver[5] and Merja Penttilä[1]. [1]VTT Technical Research Centre of Finland, Finland. [2]University of Helsinki, Finland. [3]European Bioinformatics Institute, UK. [4]Centre for Biological Sequence Analysis, Denmark. [5]University of Manchester, UK

Recent efforts to sequence fungal genomes have resulted in a greatly improved understanding of several individual species. However, little has been done to gain a comprehensive comparative view to fungal genomes. We have used TRIBE-MCL protein clustering and InterproScan protein domain analysis to compare the genomes of *Pezizomycotina* (filamentous *Ascomycota*) and *Saccharomycotina* (yeasts) species in a large data set of 33 fungal genomes. The protein clusters with interesting phylogenetic distributions were characterised with Funcat gene classifications and Protfun protein function predictions. We have also set up a GBrowse based web interface that enables easy browsing of our data. Our analysis indicates that in *Pezizomycotina* protein clusters related to plant biomass degradation and secondary metabolism are the only ones with multiple proteins from each species. *Pezizomycotina* have also interesting expansions of transcription factor and chromatin mediated gene expression regulation related clusters. In contrast *Saccharomycotina* specific protein clusters are enriched in functions related to transcription and mitochondrion.

579. Dominance *in absentia* – phantom *rec*$^{+}$ genes. F.J. Bowring, P.J. Yeadon and D.E.A. Catcheside. School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, South Australia, Australia

***rec-1*$^{+}$, *rec-2*$^{+}$ and *rec-3*$^{+}$ are dominant *trans*- acting genes that suppress meiotic recombination in specific regions of the *Neurospora crassa* genome. As an example, up to 1% of progeny from a *rec-2* by *rec-2* cross experience recombination at *his-3* but this falls to about 0.005% when one or both parents carries *rec-2*$^{+}$. Our attempt to clone *rec- 2*$^{+}$ has encountered**

substantial difficulty and the main reason for this has recently become obvious – *rec-2*$^+$ does not exist! Although approximately 10kb of the *rec-2*$^+$ chromosome is replaced with a unique 3kb stretch in *rec-2* strains, putting *rec-2*$^+$ DNA into *rec-2* strains fails to yield a *rec- 2*$^+$ phenotype. With the discovery of meiotic silencing by unpaired DNA (MSUD), we wondered whether *rec-2*$^+$ might simply appear dominant in *rec-2*$^+$*/rec-2* heterozygotes because it deprives *rec-2* of an opportunity to pair during meiosis. This appears to be the case. Disabling MSUD in *rec-2*$^+$*/rec-2* heterozygotes substantially increases recombination at *his- 3*. Similarly, in the absence of MSUD the amount of recombination at *his-1* in *rec-1*$^+$*/rec- 1* heterozygotes is indistinguishable from that in *rec-1* homozygotes. We are currently assessing whether *rec-3*$^+$ behaves similarly. Thus, rather than *rec*$^+$ genes producing suppressors of recombination it now appears likely that the products of *rec-1*, *rec-2* and possibly *rec-3* act to promote recombination.

580. Cis- and trans-splicing of Penicillium PRP8-inteins in *Escherichia coli.* Skander Elleuche*, Nicole Nolting and Stefanie Pöggeler. Department of Genetics of Eukaryotic Microorganisms - Institute of Microbiology and Genetics Georg-August-University Göttingen, Germany

Inteins are selfish genetic elements that excise themselves from the host protein during post translational processing, and religate the host protein with a peptide bond. As well as excising themselves, many large inteins also include site-specific homing endonucleases, which can initiate a gene conversion event to replicate the intein coding sequence into an intein-less allele. The first identified PRP8 intein was found within the *prp8* gene of the basidiomycete *Cryptococcus neoformans*. The *prp8* gene encodes a highly conserved mRNA splicing protein found as part of the spliceosome. Meanwhile allelic PRP8 inteins have been identified in several species of filamentous ascomycetes and basidiomycetes. In the study presented here, selected members of the ascomycetous genus Penicillium were investigated for the presence of inteins inside the PRP8 protein. We were able to identify PRP8 inteins in *P. expansum*, *P. vulpinum* and *P. chrysogenum*, but not in *P. thomii*. The Penicillium PRP8 inteins are inserted in the same position as the inteins found in the PRP8 protein of the basidiomycete *C. neoformans* and the ascomycetes *Aspergillus nidulans* and *Aspergillus fumigatus*, respectively. We demonstrated that the Penicillium PRP8 inteins undergo autocatalytical protein splicing when heterologously expressed in a model host protein in *E. coli*. In addition, we produced two-piece split intein versions of the *P. chrysogenum* PRP8-intein capable of protein trans-splicing.

581. Why accurate taxonomy is urgently needed in the age of genomics – A case study in downy mildews (Peronosporaceae). Marco Thines. University of Hohenheim, Institute of Botany 210, 70593 Stuttgart, Germany, thines@uni-hohenheim.de

First molecular phylogenies of the downy mildews were published in 2002. These resulted in numerous name changes, the merging and splitting of genera and also in a lot of confusion. As the genomic era approached, there was the desire to sequence some of these organisms and probable targets were found amongst the pathogens used in model systems. Therefore, the pathogen on *Arabidopsis thaliana* was chosen a candidate for sequencing, believing that it represented a variety of the economically important downy mildews on *Brassica* species. But this was misleading, because the species formerly known as *Peronospora parasitica* is not at all parasitic to a wide range of Brassicaceae, as presumed by numerous authors, but restricted to the type host, *Capsella bursa- pastoris*. This fact could have been known, if the taxonomic work of the early 20th century had been considered. Due to some reviews in later years, who contested the small species concept, without thorough investigations, the value of these studies was not recognized. In fact the pathogen on *Arabidopsis* should be known as *Hyaloperonospora arabidopsis* and the one on *Brassica* as *H. brassicae*. Although the genome of *H. arabidopsis* will most certainly give important insights on many aspects of the downy mildews, it will only have limited use in understanding the important pathosystems on Brassicaceae as virulence factors might be hard to compare.

582. Further development of the Homobasidiomycete Toolkit, including transformation, marker gene expression, and gene silencing technologies in the basidiomycetes *Coprinus cinereus* and *Agaricus bisporus.* M N Heneghan[1], C Burns[1], A Costa[2], K Burton[2], M P Challen[2], P R Mills[2], A Bailey[1], Gary D Foster [1]. [1]School of Biological Sciences, University of Bristol, Woodland Rd, Bristol, BS8 1UG, UK. [2]Warwick HRI, Wellesbourne, Warwick CV35 9EF, UK Email : Gary.Foster@bristol.ac.uk

We have further developed a basidiomycete Toolkit through the development of new versatile vectors, with the series containing a wide range of genes and regulatory sequences, which also allow for the rapid exchange of elements of interest. This Toolkit allows the investigation and development of a wide range of techniques, such as transformation, marker gene expression and gene silencing technologies. Post Transcriptional Gene Silencing (PTGS) methods such as RNAi have emerged as exciting new technologies in the repression of gene expression. In this report, we describe the development of gene silencing technologies within *Agaricus bisporus* and *Coprinus cinereus* as well as extending the range of marker genes available for use within the basidiomycetes. Using *C. cinereus* we describe the utilization of green fluorescent protein (GFP) in the evaluation of three different silencing cassettes, both in terms of ease of construction and

in silencing capabilities. All three constructs, untranslatable sense orientated, antisense and hairpin, were transformed into a GFP expressing *C. cinereus* strain. Lines exhibiting complete silencing, and strains exhibiting partial repression of GFP were recovered from transformations with all three cassettes. In *A. bisporus* we chose the endogenous serine protease gene as a targeted for silencing. Serine protease has been implicated in post-harvest and age-related senescence of sporophores. On harvesting, mushrooms degenerate rapidly to give browned caps and loss of texture in the fruit body, and such problems can dramatically reduce sale ability of the mushrooms. Silenced lines have been generated and these show a range of biological effects depending on the degree of silencing. Data on these studies will be presented. Suppression of genes involved in these pathways could increase mushroom shelf-life and profitability for mushroom growers, or help to further elucidate the complex biochemical pathways involved in post-harvest degradation. Gene silencing would appear to be an effective tool for the study of gene function in these fungi, and is of particular use where the dikaryotic or diploid nature of species precludes effective gene disruption. The new versatile vectors are now available as a resource to all researchers in this field.

583. Induction of programmed cell death in the filamentous fungus *Neurospora crassa.* Ana Castro[1,2] Catarina Lemos[1,2] Louise Glass[3] and Arnaldo Videira[2,3,4] .[1]Universidade Fernando Pessoa, Porto, Portugal. [2]IBMC - Instituto de Biologia Molecular e Celular, Porto, Portugal. [3]Department of Plant and Microbial Biology, UC-Berkeley, United States. [4]ICBAS - Instituto de Ciências Biomédicas de Abel Salazar, Porto, Portugal

Apoptosis is a type of genetically and physiologically controlled cell death that is associated with a series of stereotypical cytological changes. These include chromatin condensation, loss of plasma membrane asymmetry resulting in phosphatydylserine externalization (PS), DNA fragmentation, activation of caspase activity and increased production of reactive oxygen species. Mitochondria are vital organelles that perform a variety of fundamental functions ranging from the synthesis of ATP through to being intimately involved in programmed cell death (PCD). Numerous studies implicate mitochondria as key regulators of apoptosis. During its early stages, several pro-apoptotic proteins are released from the mitochondrial intermembrane space into the cytoplasm, where they bind and activate protein targets that promote later apoptotic events. The aim of our work is to establish Neurospora crassa as a model organism to study PCD. Thus, apoptosis was induced in wild type fungal cells after treatment with phytosphingosine (PHS), as detected by loss of viability and expression of cytological markers characteristic of apoptosis, such as chromatin condensation, DNA fragmentation and phosphatidylserine externalization. Interestingly, selected mutants of respiratory chain complex I respond differently to the same PHS treatment. These mutants exhibit an increased survival rate when compared with the wild type strain. The overall gene expression in PHS-induced PCD is also being analysed.

584. Farnesol signal is integrated through the RAS – Adenylate Cyclase – PKA pathway in *Candida albicans.* A. Piispanen, A. Davis-Hanna, D. Hogan. Department of Immunology and Microbiology, Dartmouth Medical School, Hanover, NH 03755, USA

***Candida albicans*, a fungus that is both a benign commensal and an important human pathogen, changes its morphology between yeast, pseudohyphal and hyphal forms in response to different extracellular stimuli. The regulated changes between yeast and filamentous forms are mediated by well-known signal transduction cascades including the Ras1-Adenylate Cyclase-Protein Kinase A pathway, however, the direct mechanism(s) by which stimuli trigger these pathways has not been fully elucidated. *C. albicans* morphogenesis is further modulated by extracellular chemical signals such as farnesol, which is produced by *C. albicans* itself, and bacterial signaling molecules such as 3-oxo-C12-homoserine lactone (3OC12HSL). We have evidence that farnesol and 3OC12HSL prevent the induction of the Ras1-AC-PKA signaling cascade. Furthermore, our analyses indicate that farnesol and 3OC12HSL likely impact this pathway through effects that are independent of Ras1 protein activation. We have found that farnesol affects key regulatory proteins under hypha-inducing conditions, but not yeast-inducing conditions, and biochemical analyses have led us to propose that farnesol affects post-translational modification events that normally occur during morphological transitions in *C. albicans*. These studies will not only provide insight into novel ways in which Ras1-AC-PKA is controlled during morphogenesis, but will bring us closer to understanding how fungi integrate environmental cues and extracellular chemical signals, such as farnesol, to modulate their behavior in multicellular communities.**

585. Susceptibility of strains of *Candida* spp. and *Cryptococcus neoformans* strains to natural compounds and antifungal drugs. Natália Faria[1], Jong Kim [2], Bruce Campbell[2] and Luz Martins[1]. [1]Instituto de Higiene e Medicina Tropical, Rua da Junqueira 96, 1349-008 Lisboa, Portugal.[2]Plant Mycotoxin Research, USDA, Albany, CA 94710 USA.

We examined differential susceptibility of pathogenic yeasts, using 3 ATCC strains of *Candida* and 250 strains of *Cryptococcus neoformans* collected from Portuguese patients, to a number of phenolic natural compounds. Preliminary tests revealed a number of promising candidate compounds. The fungicidal activities of the most potent compounds were determined based on 50% Minimum Inhibitory Concentrations (MIC_{50}). We then examined co-application of the most active phenolics with the antifungal drugs, Amphotericin B, Fluconazole, and Itraconazole, at MIC_{50} levels. Targeting

oxidative stress-response systems by the co-applied phenolics elevates fungicidal activity of the drugs. Adding safe, natural phenolic compounds to drug treatments against pathogenic yeasts could reduce dosage levels of and inhibit the potential for development of resistance to antifungal drugs. E-mail: natalia.faria@clix.pt

586. Update from the Fungal Genetics Stock Center: riding a rising tide. Kevin McCluskey, Sheera Walker, Aric Wiest and Mike Plamann. School of Biological Sciences, University of Missouri- Kansas City.

The FGSC has continued to grow as more organisms enter the post-genomics era. We have added over 2500 *Neurospora crassa* knock- out strains to the collection. To facilitate the use of these strains, we are arraying them in 96 well format and have investigated techniques to make working with arrayed mutants more straightforward.

The FGSC has also received fosmid libraries from the BROAD institute, emphasizing organisms that are either difficult to manipulate (such as Batrachochytrium) or for which a permit would be required (such as Magnaporthe), or both (Puccinia). The FGSC has also begun to distribute materials to work with fungi including Vogels' salt solution, silica gel blanks and, as part of the Neurospora Functional Genomics Program, microarray slides.

The level of activity in the fungal genetics community has contributed to increases in distribution of materials from the FGSC. After a few years of declining distribution, the FGSC has distributed record numbers of strains in the last two years. These include knock-out mutants, genome strains and clones from the genome programs. Hopefully the FGSC is not just riding the rising tide, but contributing to the rise.

The FGSC is supported by grant 0235887 from the US National Science Foundation and by National Institutes of Health Grant P01 GM068087.

587. Deleting *Aspergillus nidulans* checkpoint regulators in an undergraduate molecular genetics course. Steve James. Biology Department, Gettysburg College, Gettysburg PA sjames@gettysburg.edu

Recent advances in gene targeting and fusion PCR technology make it feasible for undergraduates to design, perform, and characterize gene deletions in filamentous fungi as part of a semester-long laboratory course. For the past two years, undergraduates in the Molecular Genetics course at Gettysburg College have deleted *Aspergillus nidulans* genes involved in cell cycle checkpoint control. Working in pairs, students applied bioinformatic tools to design a set of six PCR primers for creating a gene replacement construct. They used these primers in three-way fusion PCR to join the *A. fumigatus pyrG* selectable marker with sequences flanking the target gene. In subsequent steps, students (1) generated *pyr+* transformants in strains bearing a deletion of the KU70 homolog, (2) isolated genomic DNA and performed Southern blots using *pyrG A.f.* as a probe, (3) tested their targeted deletions for increased sensitivity to genotoxic agents, and (4) amplified and cloned the coding region into an *alcA*-based overexpression vector. Students completed this capstone experience by writing an as-for-publication paper in which they used bioinformatic tools and current literature to integrate their findings with the broader fields of cell cycle regulation and DNA damage checkpoint control. As well as providing rich opportunities for original, investigative research, this course serves as a wellspring of new projects for students wishing to continue into independent research. (Supported by Gettysburg College)

588. Analysis of the genomic ploidy of *Phytophthora infestans* by flow cytometry and fluorescence *in situ* hybridization. Mami Kaneko, Erika Arimoto, and Masatoki Taga. Department of Biology, Faculty of Science, Okayama University, Okayama, Japan. mtaga@cc.okayama-u.ac.jp

Occurrence of various polyploidy and aneuploidy in natural populations is a remarkable feature of the genome of *P. infestans*, which is regarded as one of the major drives for the evolution of this fungus. So far, analysis of the ploidy of *P. infestans* has been done with classical methods such as conventional meiotic cytology and microspectrophotometry combined with Feulgen staining. In this study, we applied flow cytometry (FCM) and fluorescence *in situ* hybridization (FISH) to ploidy analysis. In FCM, more than 20 Japanese and Dutch isolates were used. Either fixed encysted zoospores or fresh nuclei prepared from chopped mycelia were stained with PI to measure their nuclear DNA contents using nuclei from *Arabidopsis thaliana* leave cells as references. Genome size of a standard strain was estimated to be ca. 230 Mb and it was supposed to be diploid. While tri- and tetraploid strains were previously reported to be prevalent in Europe and Japan (Tooley and Therrien, 1991), the data from FCM suggested that all isolates analyzed here were not tetraploid, but either diploid or triploid with slight variation in chromosome number. In FISH, the number of homologous chromosomes per interphase nucleus was examined using rDNA and single copy genes as probes. Preliminary experiments showed that triploid isolates have an additional homologous chromosome and the size of their nuclei is significantly larger compared with diploid isolates.

589. A prion-like phenotype confers glucosamine resistance in *S. cerevisiae.* Jessica C. Brown[1] and Susan Lindquist[1,2]. [1]Dept. of Biology, MIT, Cambridge, MA [2]Whitehead Institute, Cambridge, MA

Several well-characterized fungal proteins are capable of acting as prions. Prions are proteins capable of multiple self-propagating conformations, each with different activities. Different prion conformers in cells with identical genotypes result in multiple different phenotypes. The ability of particular proteins to form prions is found from *S. cerevisiae* to *C. albicans*. Resistance to the non-hydrolyzable glucose analog D-(+)-glucosamine in S. cerevisiae is inherited in a non-Mendelian, prion-like pattern called [GAR+]. [GAR+] appears spontaneously at a high frequency (~2 in 10^4 cells), and segregates in a non-Mendelian 4 [GAR+] to 0 [gar-] pattern following meiosis. [GAR+] can be inherited by cytoplasmic transfer without nuclear exchange. Also, [GAR+] can be converted to [gar-] by altering levels of molecular chaperones (i.e. "cured"). The [GAR+] casual agent is unknown. However, [GAR+] cells show pleiotropic biological properties, including altered cell wall structure and an ability to outcompete [gar-] cells when grown in a mixture of glucose and other carbon sources. Gene expression analysis showed little difference between [gar-] and [GAR+] cells with one exception: Hexose Transporter 3 was 35 fold downregulated in [GAR+] compared to [gar-]. The GAR determinant is therefore in the pathway downstream of glucose sensors Rgt2p/Snf3p. A member of this pathway strongly induces the [GAR+] phenotype but is not required for [GAR+] propagation. [GAR+] thus requires separate agents for its induction and propagation. This differs from other prions, which require only one factor for both propagation and induction.

590. Nitrate reduction by *Aspergillus nidulans* under anaerobic conditions. Naoki Takaya, Kazuto Takasaki, Tatsuya Fujii, and Tatsuya Kitazume. Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan. e-mail; ntakaya@sakura.cc.tsukuba.ac.jp

Nitrate is one of the preferable nitrogen sources for filamentous fungi although it is reduced to ammonium and excreted to the culture medium under anaerobic conditions. We identified the *Aspergillus nidulans* genes involved in this process by analyzing fungal mutants. The results showed that assimilatory nitrate and nitrite reductases (the gene products of *niaD* and *niiA*) were essential for reducing nitrate and for anaerobic cell growth. We also found that ethanol oxidation is coupled with nitrate reduction and catalyzed by alcohol dehydrogenase, coenzyme A (CoA)-acylating aldehyde dehydrogenase, and acetyl CoA synthetase (Acs). The production of Acs requires a functional facA gene that encodes Acs. We purified the gene product of *facA* (FacA) from the fungus to show that the fungus acetylates FacA on its lysine residue(s) specifically under the anaerobic conditions to regulate its substrate affinity. Acetylated FacA had higher affinity for acetyl CoA than for acetate, and is responsible for ATP synthesis under the anaerobic conditions. These results showed that the reaction is of significant for a fermentation mechanism. Exogenous ammonium represses expression of *niaD*. Under anoxic conditions, however, *A. nidulans* expressed *niaD* even in the presence of ammonium. Since the fungus produces enough amounts of ammonium (~ 10 mM) that could repress expression of the niaD gene, the anoxic expression mechanism of *niaD* in the presence of ammonium is physiologically significant for the fungus to produce nitrate reductase which is critical for the fungus ferments ammonium.

591. The multi-KH domain protein Khd4 effects pathogenicity by regulating mRNA stability and/or translation in *Ustilago maydis*. E. Vollmeister, P. Becht, S. Baumann and M. Feldbrügge. Max-Planck-Institute for terrestrial Microbiology, Karl-von-Frisch-Str., 35043 Marburg, Germany, evelyn.vollmeister@mpi-marburg.mpg.de

***Ustilago maydis* is the causative agent of corn smut disease. Pathogenic development of this basidiomycete depends on various morphological transitions, for instance the formation of conjugation hyphae or infectious filaments. Since the *khd4* deletion strain is affected in these developmental steps the multi-KH domain protein Khd4 plays an important role in these processes. However, the molecular function of Khd4 is at present unknown. Here we show that Khd4 is a sequence-specific RNA-binding protein. Using the yeast three-hybrid system we defined a Khd4 response element (KRE), AUACCC, which is necessary for Khd4 binding *in vivo*. Since Khd4 accumulates after stress induction in cytoplasmic particles (processing bodies) which are thought to be sites of mRNA degradation and/or mRNA storage in lower eucaryotes we propose a function of Khd4 in control of mRNA stability and/or translation. Currently, we are combining microarray analysis with a proteomic approach to determine target mRNAs of Khd4.**

592. Development of a genetic map for *C. lindemuthianum*. June Simpson. Cinvestav, Genetic Engineering, Guanajuato MX.

A framework genetic map was developed for the fungal pathogen *Colletotrichum lindemuthianum*, the causal agent of anthracnose of common bean *(Phaseolus vulgaris L.)*. This is the first genetic map for any species within the family Melanconiaceae and the genus Colletotrichum and provides the first estimate of genome length for *C. Lindemuthianum*. The map was generated using 106 haploid F1 progeny derived from crossing two Mexican *C. lindemuthianum* isolates differing in two avirulence genes *(AvrclMex and AvrclTO)*. The map comprises 165 AFLP markers covering 1897 cM with an average spacing of 11.49 cM. The markers are distributed over 19 major linkage groups containing between 5 and 25

markers each and the genome length was estimated to be approximately 3241 cM. The avirulence genes *AvrclMex and AvrclTO* segregate in a 1:1 ratio supporting the gene for gene hypothesis for the incompatible reaction between *C. lindemuthianum* and *P. vulgaris,* but could not be incorporated into the genetic map. This initial outline map forms the basis for the development of a more detailed *C. lindemuthianum* linkage map which would include other types of molecular markers and allow the location of genes previously isolated and characterized in this species. This research was supported by grants from CONACyT/SAGAR no. K159-A9702 and SEP-CONACyT grant nos. 28275 and 40369-Q.

593. Much higher MMS-sensitivity of *uvsH77* among other *uvsH* mutant alleles was resulted from the existence of the second mutation, *smuH501* linked very closely to *uvsH* in *Aspergillus nidulans.* Suhn-Kee Chae and Mee-Hyang Jeon. Department of Biochemistry, Paichai University, Daejeon 302- 735, Korea. chae@pcu.ac.kr.

***smuH* (An extra copy suppressor of mutagen-sensitivity of *uvsH77*) was originally isolated as a DNA clone complementing partially the MMS-sensitivity of *uvsH77* mutant [Mol Gen Genet (1995) 248:174-181]. Molecular characterization of *smuH* has been carried out and a null mutant of *smuH* was generated. *ΔsmuH* exhibited high camptothecin (CPT)- but very slight MMS-sensitivities. *uvsH77* mutant strains also showed high sensitivity to CPT, a topoisomerase I inhibitor. We noticed, however, that the CPT-sensitivity in *uvsH77* mutants was resulted from a second mutation in the original *uvsH77* mutant strain. During the analysis of CPT-sensitivities in meiotic progenies of heterozygotic crosses of *uvsH77*, some meiotic progenies without *uvsH77* showed high CPT- but slight MMS-sensitivities, while segregants with only *uvsH77* exhibited no CPT- and much reduced MMS-sensitivities than original *uvsH77* mutants. The second mutation, called *smuH501* was shown to be a mutant allele of *smuH*. The mutation site was also identified in *smuH501*, changing the 56th lysine residue to a stop codon. So, *smuH* was redefined as "*S*econd *M*utation in *u*vs*H*77". Null mutants of *uvsH77* showed MMS-sensitivity similar to those shown in *uvsH304* and *uvsH311* mutant alleles. Synergistic interaction between *smuH* and *uvsH* were evident. *smuH501* also showed synergistic interaction with *uvsF201* encoding replication factor C. *smuH* will be discussed more in the presentation.**

594. Effect of osmotic stress on sclerotial and aflatoxin production in *Aspergillus flavus*. Rocio M Duran and Ana M Calvo. Department of Biological Sciences, Northern Illinois University, 1425 W Lincoln Hwy, DeKalb, Illinois.

Fungal cells respond to osmotic stress with molecular adaptations that result in chemical and morphological changes. We examined the effect of environmental osmotic stress on the fungus *Aspergillus flavus*. We found that hypertonic media differentially affected the formation of resistant structures, sclerotia, and the biosynthesis of the carcinogenic mycotoxin aflatoxin. While aflatoxin biosynthesis slightly increased in media with high concentration of sodium chloride or sorbitol, production of sclerotia was greatly reduced. The strong reduction of sclerotia was accompanied by an increase in colony growth and conidiophore formation. Search of the *A. flavus* genomic database allowed us to identify *AFhogA*, a gene encoding a putative homolog of the mitogen-activated protein kinase HOG1 that mediates the osmotic stress response in *Saccharomyces cerevisiae*. Additional components of this signaling pathway were also found in the *A. flavus* genomic sequence. We are further investigating the implications of this pathway in the regulation of resistant structure formation in this important plant pathogen.

595. Teaching fungal genetics at the HAN BioCentre. Christien Lokman, Nick van Biezen, Kees van den Hondel. HAN University, BioCentre, P.O. Box 6960, 6503 GL Nijmegen christien.lokman@han.nl

The HAN BioCentre is a contract research organisation of the HAN University which focuses on applied research for Small and Middle seize Enterprises (SME's) in the field of industrial microbiology. Extensive experience is present within the HAN BioCentre regarding production of (heterologous) proteins in filamentous fungi. Talented and well-motivated bachelor- and master students contribute to research projects under direct supervision of members of the research staff of the HAN BioCentre. In this way the students can already obtain experience during their study in applied research in a real working environment gaining both practical skills and earning some money. An additional advantage of the HAN BioCentre is that new technologies and knowledge that has been developed subsequently will be introduced in our Bachelor and Master programmes. In this way we will keep the programmes up-to-date. The advantage for the SME's is obvious. For reasonable costs they will get direct access to expertises and facilities of the HAN BioCentre pushing their research and development forward. Also large scale experiments, such as complicated mutant screens, are able to be carried out by a larger number of students, increasing in this way the chance of isolating mutants which meets in the best way the criteria postulated.

596. Deletion of *msh-2*: expected phenotype with some surprises. P. J. Yeadon, F. J. Bowring and D. E. A. Catcheside. School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, South Australia, Australia

Msh-2 is a highly conserved protein required for eukaryotic repair of mismatches in DNA. We have deleted the Neurospora *msh-2* orthologue in a number of strains. Of 43 octads from an *msh-2* deletion homozygote, 6 displayed non-Mendelian segregation at *his-3*, *lys-4* or *cot-1*. In all cases the marker segregated 5:3, confirming the requirement for Msh-2 in Neurospora mismatch repair. Among 192 random progeny of a mutant cross all 16 genotypes were detected, including those that arose from double or triple crossovers, extremely rare events in a wild type cross. Msh-2 may thus have an unexpected role in crossover interference. Although an *msh- 2*^{RIP} null allele was shown to increase *his-3* allelic recombination, we have found this to be true only for *his-3* alleles of different wild-type origin, suggesting Msh-2 may regulate recombination between dissimilar sequences. In contrast, preliminary data suggest crossing over in intervals flanking *his-3* is unaffected by deletion of *msh-2*, even when the sequences are known to be substantially divergent. This work was supported by a grant from the Australian Research Council.

597. A new crossover hotspot between *cog* and *his-3*? P. J. Yeadon, F. J. Bowring and D. E. A. Catcheside. School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, South Australia, Australia

Two distinct lines of evidence indicate the existence of a crossover hotspot at least 2kb from the recombination hotspot *cog*. Firstly, deletion or replacement of a 1.8 kb fragment between *his-3* and *cog* reduces crossing over in the region. Initially we thought the decline in recombination was due to lack of homology reducing the efficiency of local chromosome pairing. However, crossing over is not restored even when the same sequence is inserted into both chromosomes, so the fragment that has been replaced may include a crossover hotspot. Secondly, 150 octads have been analysed for segregation of both nutritional and molecular markers, the latter spanning 60 kb of LGI, and an excess of crossovers was found in a 200 bp interval close to the 3' end of *his-3*. We plan to identify the sequence responsible for the hotspot using deletion. This work was supported by a grant from the Australian Research Council.

598. Approach to establish a high throughput molecular genetic analysis of *Colletotrichum higginsianum* by *Agrobacterium tumefaciens*-mediated transformation. Hiroshi Terada, Kie Tsuboi, Ai Mori, Gento Tsuji, and Yasuyuki Kubo. Laboratory of Plant Pathology, Kyoto Prefectural University, Kyoto, Japan.

***Colletotrichum higginsianum* is a causal agent of anthracnose disease of *Brassicaceae* plants. This fungus has become an excellent candidate for studying fungal-plant interactions because it can infect a model plant, *Arabidopsis thaliana.* The aim of our study is to establish a high throughput molecular genetic analysis of this fungus by *Agrobacterium tumefaciens*-mediated transformation (AtMT). We have already developed AtMT for random insertional mutagenesis in *C. higginsianum*. Here we describe the attempts to develop it for targeted gene disruption. We estimated an efficiency of homologous integration for two isolated genes, *ChPKS1* and *ChSSD1*. Although AtMT provided high efficiency of transformation, homologous integration occurred only 2.4% for *ChPKS1* and 0.6% for *ChSSD1*. This result suggested that it would be impractical to isolate recombinants efficiently in *C. higginsianum*. Thus, we focused on the *Neurospora crassa mus-51* gene encoding a protein that functions in nonhomologous end-joining of DNA breaks. It was reported that the disruption strains of the gene showed a dramatic increase of homologous integration efficiency. We isolated the *mus-51* homologue *ChMUS51* gene from *C. higginsianum*, and successfully generated disruption strains. Now we are evaluating the strains for efficiency of homologous integration.**

599. The *ActA* gene of *Aspergillus nidulans* encodes a yeast *GCN20* homolog. Sun-Hee Noh, Eun-Jung Kim, Mee-Hyang Jeon, and Suhn-Kee Chae. Dept. Biochemistry and Biomed RRC, Paichai Univ. Daejeon, 302- 735 Korea. chae@pcu.ac.kr.

During the screening of rapamycin-sensitive and -resistant mutants in *A. nidulans*, we observed that rapamycin sensitivities of FGSC strains were correlated to the presence of the *ActA1* mutation causing Actidion (cycloheximide) resistance in the strains. In not a single case of more than 400 strains tested by Dr. Etta Kafer was there a separation of *ActA1* from rapamycin sensitivity. To confirm that *ActA1* caused rapamycin-sensitivity, molecular cloning of a gene complementing the mutation of an rapamycin-sensitive *ActA1* mutants was carried out by transforming the mutant with a genomic DNA library. About 8 kb-long DNA fragment was isolated from a transformant and sequenced. This DNA fragment contained only one full-size ORF and was located in position of *ActA* on chromosome III. Disruption of the cloned gene resulted in cycloheximide resistance but wild-type level sensitivity to rapamycin. A heterozyous diploid of *ΔactA* was not resistant to cycloheximide. Sequencing of the PCR product from an *ActA1* mutant with primers for the cloned gene displayed mutations at 393rd and 394th amino acid residues causing amino acid changes of EP to GT. These results indicate that the complementing clone was indeed *ActA*. The *ActA* coding region is 2,596 bp-long interrupted by 7 introns, encoding a protein with a strong similarity to Gcn20, a positive effector of eIF2-alpha kinase Gcn2 in yeast. [Supported by Korea Research Foundation]

600. Study of taxonomy and ecology of *Termitomyces* spp. Prapapan Sawhasan , Jeerapun Worapong and Taworn Vinijsanun. Department of Biotechnology, Faculty of Science, Mahidol University, Rama 6, Bangkok 10400, Thailand

Kanchanaburi forests where are located in the west region of Thailand are well known for high diversity of *Termitomyces* spp., but none has clearly understand for the taxonomy of this mushroom. It has been found that there were only 4 species out of 10 *Termitomyces* isolates, *T. robustus, T. bulborhizus,T. fuliginosus* and *T. striatus,* that were able to be identified based on morphological features. Two isolate should be classified as a novel specie because some morphologies did not match any known species. Their feature descriptions are provided. The inferred NJ phylogram of ITS1&2 and 5.8S rDNA sequences showed that 10 selected *Termitomyces* isolates were monophylatic of 2 clades that are corresponding to the size of basidiocarps.

601. Role of PmrA and Pmc1 in calcium signalling of the filamentous fungus *Aspergillus niger.* Bagar T, Benčina M. National Institute of Chemistry Slovenia, Ljubljana

Calcium is one of the most important signalling molecules in the living world. The divalent cation Ca^{2+} is used by cells as a second messenger to control many cellular processes. In filamentous fungi it has been shown to effect elongation and branching of hyphae, dimorphism, sporulation and spore germination, response to osmotic stress, cell cycle regulation, circadian rhythm and other processes.

The cytosolic level of Ca^{2+} is kept low in resting cells (50 - 300 nM in fungi), but stimulation results in the level increasing up to 1000 fold. The cell has access to two sources of Ca^{2+}: entry from the external medium and release from internal stores (organelles). Fungal cells store intracellular calcium primarily in the vacuoles but also in the endoplasmic reticulum and mitochondria. We are currently studying the role of PmrA endoplasmic reticulum Ca-ATP-ase and Pmrc1 vacuolar Ca-ATP-ase on the intracellular calcium signalling in *Aspergillus niger*. Using knock-out mutants we are evaluating the effect of these Ca-ATP-ases on the resting level of calcium in the cytoplasm and on the response to external stimuli such as mechanical perturbation, hypo-osmotic stress and high external calcium.

KMC 4/2/07

The Fourth *Aspergillus* Meeting

March 18-20, 2007
Asilomar Conference Center
Pacific Grove, California

Organized by the
***Aspergillus* Genomes Research Policy Committee**

Aspergillus Genomes Research Policy Group (AGRPG)

An *Aspergillus* Genomics workshop was held at the March 2003 Asilomar Fungal Genetics meeting. From discussions in that workshop it was obvious that our community needed to organize to fully exploit genomics resources. A provisional *Aspergillus* Genomes Research Policy Committee (AGRPC) was conscripted and charged with creating a structure for community-wide coordination and organizing an annual meeting. The First *Aspergillus* Meeting was held in Copenhagen, April 21, 2004, as a satellite meeting of the European Congress on Fungal Genetics-7. In addition to scientific presentations, bylaws were approved, community research directions were discussed and the 2004 AGPRC was elected. The name *Aspergillus* Genomes Research Policy Group was adopted for the community. The objectives of the AGRPG are: (1) Provision of an educational and discussion forum for issues pertaining to *Aspergillus* genomics, in this widest sense, for the various *Aspergillus* research communities; (2) Influencing grant making bodies and other institutions on behalf of the various *Aspergillus* research communities; (3) Coordinating research activities internationally, as and when required, to future the science base of the *Aspergillus* genus. For more information on the activities of the AGRPG and other *Aspergillus* news see our homepage at FGSC (http://www.fgsc.net/Aspergillus/asperghome.html).

2006 AGRPC:

Scott Baker (*A. niger)*, 2006-08
Pacific Northwest National Laboratory, USA; scott.baker@pnl.gov
Gerhard Braus (*A. nidulans*), 2005-07
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Paul Dyer (*A. fumigatus*), 2004-06
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Gustavo Goldman (Special Appointment), 2006
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The Fourth *Aspergillus* Meeting

March 18-20, 2007
Asilomar Conference Center

All sessions in Merrill Hall

March 18, Sunday

3:00- 6:00 Registration

6:00 ***Dinner***

7:00-9:00 ***Welcome Reception—Sponsored by Novozymes***

March 19, Monday

7:30-9:00 ***Breakfast***
9:00-9:15 **Welcome, introductions and announcements**

Session I:
9:15-10:15 **Genetics and Cell Biology** **Chair: Gerhard Braus**
Dr. Norio Takeshita, Univ. of Karlsruhe. Cell end factors in *A. nidulans*

Dr. Jae Hyuk Yu, Univ. of Wisconsin. GPCRs, RGSs and velvet-like proteins

Dr. Kerstin Helmstaedt, Univ. of Göttingen. The nuclear migration protein NUDF associates with BNFA and NUDC at spindle pole bodies in *Aspergillus nidulans*.

10:15-10:45 ***Coffee Break –Sponsored by Monsanto***

Session II:
10:45-11:45 **Phylogenetics/Evolutionary Biology** **Chair: Paul Dyer**

Dr. Arun Balajee, Centers for Disease Control and Prevention, Atlanta. MLST and the molecular phylogeny of the *Aspergillus* section *Fumigati*.

Dr. Peter J. Cotty, United States Department of Agriculture, University of Arizona. Vegetative incompatibility in *Aspergillus flavus*.

Dr. Rolf F. Hoekstra, Wageningen University.
Experimental means to study evolutionary processes within *Aspergillus*

12-1:00 ***Lunch***

Session III:

1:00-2:00 **Annotation, Comparative Genomics, Databases**
Chair: Masayuki Machida

Dr. Jennifer Wortman, The Institute for Genomic Research. Comparative genomics as a tool for annotation improvement and analysis of related *Aspergillus* genomes.

Dr. Gavin Sherlock, Candida Database, CGD. Using GBrowse to visualize multiple genome assemblies.

Drs. Jennifer Wortman, Steve Oliver, Dave Ussery, Jaap Visser and Cees van den Hondel. The *A. nidulans* annotation project: a global community effort.

2:00-2:30 **Community Directions Discussion**
Moderator: Michelle Momany

2:30-2:45 ***Coffee Break –Sponsored by Monsanto***

Session IV:

2:45-5:00 **Talks selected from Abstracts Chair: Gary Payne**

Dr. Özgür Bayram, Georg August University, Institut of Microbiology & Genetics Goettingen. The *Aspergillus nidulans* F-box Protein Project.

Dr. Jakob B. Nielsen, Technical University of Denmark, CMB, BioCentrum-DTU Kgs. Lyngby. A transiently disrupted non-homologous end joining pathway in *Aspergillus nidulans* allows simple and efficient gene targeting.

Dr. Peter J. Punt, TNO Quality of Life, Microbiology Zeist. New screening approaches for fungal strain development.

Dr. Natalie D. Fedorova, The Institute for Genomic Research, Microbial Genetics, Rockville, MD. Comparative analysis of secondary metabolism gene clusters from two strains of *Aspergillus fumigatus* and closely related species *Neosartorya fischeri* and *Aspergillus clavatus*.

Dr. Ronald de Vries, Utrecht University Microbiology, Utrecht. Colonies from the filamentous fungus *Aspergillus niger* are highly differentiated in spite of cytoplasmic continuity.

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Dr. William Steinbach, Pediatrics, Molecular Genetics, and Microbiology, Duke University Medical Center. The benefits and real future of calcineurin inhibition against invasive aspergillosis.

Dr. William C. Nierman, The Institute for Genomic Research. A pilot project for a high throughput *Aspergillus fumigatus* mutant strain Resource

Dr. Steven Harris, The University of Nebraska. Proposed tiling project

5:15-6:00 **Pontecorvo Lecture: Joan Bennett, Rutgers University**
"On being Aspergillus-o-centric: From girl geek to fungus freak"

6:00-7:00 ***Dinner***

7:00-10:00 **Posters and drinks**
Poster session and outstanding student poster sponsored by DSM

March 20, Tuesday

7:30-9:00 ***Breakfast***

9:00-9:15 Elections

Session V:

9:15-10:15 **Genomics/Proteomics/transcriptomics/metabolomics updates**
Chair: Gustavo H. Goldman

Dr. Manda Gent, Faculty of Life Sciences, Manchester University A comparison of the expression of secreted hydrolases in *Aspergillus fumigatus* and *Aspergillus nidulans* under phospholipid-rich conditions

Dr. Olaf Kniemeyer, Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie Hans-Knöll-Institut. Comparative proteomics of the human-pathogenic fungus *Aspergillus fumigatus*

Dr. Jens Frisvad, Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark. The exometabolomics of *Aspergilli*

Dr. Noel van Peij, DSM. *A. niger* genome update

10:15-10:30 ***Coffee Break–Sponsored by Monsanto***

Session VI:

10:30-11:30 **Industrial Applications** **Chair: Scott Baker**

Dr. Ken Bruno, Chemical & Biological Processes Development Group, Pacific Northwest National Laboratory. Cell Biology and Citric Acid Production in *Aspergillus niger*.

Dr. Mikael Rørdam Andersen, Technical University of Denmark. Metabolic network driven interpretation of transcription data from *Aspergillus* cell factories.

Dr. Adrian Tsang, Concordia University. *Aspergillus niger* as a host for global expression of secreted proteins.

11:30 **Announce election results and take any further discussion**
Announce winner of DSM student poster prize

12:00-1:00 ***Lunch***

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Poster Abstracts

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CELL BIOLOGY

1. The *Aspergillus nidulans cetA* and *calA* genes are involved in conidial biogenesis and germination. Ravit Balaish, Chaim Sharon, Emma Levdansky, Shulamit Greenberg, Yana Shadkchan and Nir Osherov[1] Department of Human Microbiology, Sackler School of Medicine, [1]Tel-Aviv University Tel-Aviv, Israel.E-mail: nosherov@post.tau.ac.il

Recently, we characterized the *cetA* (AN3076) gene, whose transcript is highly expressed in dormant conidia of *A.nidulans*1,2. CetA, is similar to plant thaumatin-like (TL) genes encoding proteins which have an antifungal activity. We found that the cetA transcript is rapidly translated during early germination and secreted into the culture medium. It was also found that cetA transcription is subject to glucose-mediated carbon catabolite repression (CCR) and is activated by the PKA pathway. However, *cetA* gene-disruption showed no obvious phenotypic growth defects, as compared to the wild-type strain. *A. nidulans* contains an additional cetA-like gene, *calA* (AN7619), whose transcript is not expressed in dormant conidia but only during germination. We hypothesized that *cetA* and *calA* are redundant and that deletion of both genes may reveal their function. We therefore prepared knockout *cetA, calA* and *calA/cetA A. nidulans* strains and analyzed their phenotype. The mutants in which *calA* and *cetA* were deleted were phenotypically identical to the wild-type strain. In contrast, the *cetA/calA*-double mutant showed a lethal phenotype. Most of the conidia in the double mutant were completely inhibited in germination. Many collapsed and underwent autolysis. A few showed abnormal germination characterized by short swollen hyphae, and abnormal hyphal branching. Furthermore, the ungerminated conidia contained a single condensed nucleus suggestive of a defect in initiating the cell life-cycle (mitosis did not occur). This is the first study to analyze the function of the novel *cetA/calA* family of thaumatin-like genes and their role in the germination of *A. nidulans* conidia. We show that *cetAp* and *calAp* are redundant proteins that together play an essential role in the development of the conidial cell wall.
1)Osherov et al. FGB 37:197-204, 2002. 2)Greenstein et al. FGB 43:42-53, 2006.

2. Characterization of the *Aspergillus nidulans* septin *AspA/Cdc11*. Rebecca Lindsey, Susan Cowden and Michelle Momany* Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA lindsey@plantbio.uga.edu

The septin proteins form filamentous rings at the mother-bud neck in yeast. Septins are found in microsporidia, fungi, and animals and are absent from plants. In addition to their original role in cell division, septins have been shown to have roles in cytoskeletal organization, coordination of nuclear division and trafficking across membranes. In *Aspergillus nidulans* there are five septins *aspA/cdc11, aspB/cdc3, aspC/cdc12, aspD/cdc10*, and *aspE*. We have conducted studies on the *A. nidulans* septin *aspA/cdc11*. The null mutant of *aspA/cdc11* is viable and shows uncoordinated germ tubes, hooked hyphae, split tips, hyperbranching, disorganized conidiophores and hypersensitivity to the actin depolymerizer Cytocholasin A. Localization of AspA/Cdc11-GFP is dynamic. AspA/Cdc11-GFP is visible as a spot on conidia, throughout the cortex in swelling conidia, at the polarizing surface when germ tubes emerge, at septa in germ tubes and branches, and as filaments at tips and in early branches. In *delta aspA/cdc11* SepA-GFP and BimG-GFP localization are lost, but TubA- GFP localization is normal. Our results suggest that AspA/Cdc11 interacts with actin as well as the formin, SepA and the protein phosphatase BimG, possibly in the growing hyphal tip.

3. Characterization of Protein *O*-mannosyltransferases in *Aspergillus nidulans*. Thanyanuch Kriangkripipat and Michelle Momany* Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA tkriang@plantbio.uga.edu

Protein *O*-mannosyltransferases (PMTs) are found in bacteria, fungi, and animals but are not present in plants. In fungi, PMTs are divided into three subfamilies, PMT1, PMT2 and PMT4 and each species has 3-7 PMTs. *Aspergillus nidulans* possesses three PMTs, Pmt1, Pmt2 and Pmt4. Single *pmt* deletion mutants are viable. Each Δ*pmt* mutant exhibits different phenotypes when characterized by growth at different temperatures, morphology and sensitivity to chemicals disturbing cell wall synthesis. Double mutants show additive phenotypes. The Δ*pmt1* mutant has hyphal tip lysis and produces aberrant conidiophores at 42^{O}C. The Δ*pmt2* mutant cannot send out germ tubes at 42^{O}C. The Δ*pmt4* mutant has swollen hyphae and produces aberrant conidiophores at 42^{O}C. The Δ*pmt1*Δ*pmt2* double mutant is viable and has additive phenotypes of Δ*pmt1* mutant and Δ*pmt2* mutant. The Δ*pmt2*Δ*pmt4* double mutant is viable but very sick and forms a microcolony only when an osmotic stabilizer is added to the medium. Lower temperatures and osmoticum can partially restore wildtype hyphal growth and conidiation of these Δ*pmt* mutants except for the Δ*pmt2*Δ*pmt4* double mutant. Our results suggest that protein *O*-mannosylation is important for cell wall integrity of *A. nidulans*.

4. Characterization of the *Aspergillus nidulans* septin mutant *ΔaspB/cdc3*. Yainitza Rodriguez and Michelle Momany Department of Plant Biology, University of Georgia, Athens, Georgia 30602 USA *yrodriguez@plantbio.uga.edu*

Septins are filament forming P-loop GTPases found in microsporidia, fungi and animals. Septins play important roles in a variety of processes such as cellular and nuclear division, membrane trafficking and organization of the cytoskeleton. This family of proteins was first discovered in a screen for temperature sensitive cell cycle mutants in *Saccharomyces cerevisiae*. In yeast, septins form filamentous rings at the mother-bud neck which are necessary for completion of cytokinesis and normal morphogenesis. In *Aspergillus nidulans* there are five septins *aspA/cdc11*, *aspB/cdc3*, *aspC/cdc12*, *aspD/cdc10*, and *aspE*. The *A. nidulans* septin *aspB/cdc3* was previously shown to localize at septation and branching sites and at interface layers of the conidiophore. This gene was reported to be essential. We have now found that deletion of *aspB/cdc3* is not lethal, but causes severe defects in asexual reproduction and aberrant morphology in several developmental stages. The mutant shows emergence of multiple germ tubes, hyperbranching and hooked branches and disorganized conidiophores. *ΔaspB/cdc3* also shows sensitivity to calcoflour, but not benomyl or cytochalasin A, which suggests that the mutant phenotype is not directly associated with the actin or microtubule cytoskeleton, but perhaps with cell wall stability.

5. The Nuclear Migration Protein NUDF Associates with BNFA and NUDC at Spindle Pole Bodies in *Aspergillus nidulans*. Kerstin Helmstaedt, Karen Meng, Silke Busch, Özgür Bayram, Oliver Valerius and Gerhard H. Braus. Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstr. 8, D-37077 Göttingen, Germany. Tel. +49-551-3919693, Fax +49-551-393820, khelmst@gwdg.de

In *A. nidulans*, nuclear migration depends on microtubuli, the motor dynein and nuclear distribution proteins like NUDF. Applying tandem affinity purification, we isolated a unique NUDF-associated protein, which we named BNFA (Binding of NUDF). An *A. nidulans bnfA* deletion strain did not show a *nud* phenotype indicating that a protein with redundant function might exist. A GFP-BNFA fusion localized to spindle pole bodies (SPBs) throughout the cell cycle. This position depended on NUDF, since in a *nudF6* strain BNFA localized mainly to dots in the cytoplasm. In a yeast two-hybrid screen using BNFA as bait, we found that BNFA is a dimer and that a link might exist to the septation signalling pathway. In a candidate approach, we analysed the putative NUDC-NUDF interaction in *A. nidulans*. Although NUDC-GFP alone was localized to immobile dots at the cortex, we found a direct interaction between NUDF and NUDC in yeast two-hybrid experiments, which depended on NUDF's WD40 domain. Applying bimolecular fluorescence complementation microscopy, we showed that *in vivo* NUDF and NUDC interact also at spindle pole bodies throughout the cell cycle and at immobile dots at the cortex.

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6. Analysis of two cell end marker proteins, TeaA and TeaR, in *Aspergillus nidulans*. Norio Takeshita, Sven Konzack, Yuhei Higashitsuji and Reinhard Fischer. Applied Microbiology, University of Karlsruhe, Germany.

The interplay of the actin and the microtubule (MT) cytoskeleton in polarized growth of fungi has recently been revealed. In *Schizosaccharomyces pombe*, Tea1 is a key protein in this process. Tea1 is transported to the plus ends of MTs by the kinesin Tea2, and is delivered to cell ends by hitchhiking with the growing MTs. Mod5, which is posttranslationally modified by prenylation, anchors Tea1 at the cell ends. These three proteins were indentified by screening for polarity mutants. At the cell ends, Tea1 recruits formin which initiates actin assembly and the establishment of cell polarity. Tea1 and Tea2 homologues were identified in the *Aspergillus nidulans* genome (named TeaA and KipA), whereas Mod5 could not be identified due to sequence similarity. *kipA* mutants showed mislocalization of the Spitzenkorper and hence curved hyphae. GFP-KipA accumulated at the MT plus ends. *teaA* mutants showed a similar but not identical phenotype to *kipA* mutants. GFP-TeaA localized to one point in the apex of hyphal tips. To test whether the function of *S. pombe* Mod5 was conserved, we searched the *A. nidulans* genome for proteins with a C-terminal prenylation motif (CAAX). From 22 identified proteins one (536 amino acids) was likely to serve a TeaA-anchorage function. We named it TeaR. *teaR* mutants indeed displayed a phenotype similar to the *kipA* mutant. GFP-tagged TeaR localized to the membrane at hyphal tips. Their putative interactions will be analyzed.

7. Secondary metabolism and sporulation signalling in *Aspergillus nidulans*. Olivia Márquez[1], Angel Trigos[1], J. Luis Ramos[2], Gustavo Viniegra[3], Holger Deising[4], Nallely Cano[2] and Jesús Aguirre[2]*. [1]Universidad Veracruzana, México. [2]Universidad Nacional Autónoma de México, México. [3]Universidad Autónoma Metropolitana, México. [4]Martin-Luther University Halle-Wittenberg, Germany. *E-mail: jaguirre@ifc.unam.mx

We characterized *Aspergillus nidulans* strains carrying conditional (*cfwA2*) and null (*DcfwA*) mutant alleles of the *cfwA/npgA* gene, encoding an essential phosphopantetheinyl transferase (PPTase). We identified the polyketides shamixanthone, emericellin and dehydroaustinol, as well as the sterols ergosterol, peroxiergosterol and cerevisterol in extracts from developmental cultures. The PPTase CfwA/NpgA was required for production of polyketide compounds, but dispensable for fatty acid biosynthesis. The asexual sporulation defects of *cfwA, DfluG* and *DtmpA* mutants were not rescued by the *cfwA*-dependent compounds identified here. However, *cfwA2* mutation drastically enhanced the sporulation defects of both *DtmpA* and *DfluG* single mutants, suggesting that unidentified CfwA-dependent PKS and/or NRPS are involved in production of unknown compounds, required for sporulation. In addition, we show that *tmpA* and *tmpB* genes define two new *fluG*-independent sporulation pathways.
Supported by CONACYT-SAGARPA 2002-C01-1713, México.

8. Functional analysis of histidine-containing phosphotransmitter gene *ypdA* in *Aspergillus nidulans*. Natsuko Sato, Kentaro Furukawa, Tomonori Fujioka, Osamu Mizutani, and Keietsu Abe. Molecular and Cell Biology, Tohoku University, Sendai, Japan.

The high-osmolarity glycerol (HOG) response pathway responding to osmotic stimuli has been well studied in *Saccharomyces cerevisiae*. Sln1p-Ypd1p-Ssk1p proteins organize a two-component signalling (TCS) unit in the upstream of the HOG pathway, and negatively regulate the downstream Hog1p mitogen-activated protein kinase (MAPK) cascade. We previously revealed that a filamentous fungus *Aspergillus nidulans* possesses all counterparts of the components of *S. cerevisiae* HOG pathway. Deletion of Ypd1p, the TCS histidine-containing phosphotransmitter of *S. cerevisiae*, is known to cause lethality because of constitutive activation of Hog1 MAPK. While, *S. cerevisiae* possesses only one set of TCS unit consisted of Sln1p and Ypd1p, *A. nidulans* has been predicted to have 15 histidine kinases and some of them are thought to interact with the unique YpdA. Thus, the TCS pathway of *A. nidulans* might be more complex and robust than that of yeast . In addition, *YPD1* is essential in *S. cervisiae* but not in *Shizosaccharomyces pombe*. In the present study, in order to examine in vivo functionality of *A. nidulans ypdA* gene, we constructed an *ypdA* delta strain conditionally expressing the *ypdA* gene under the control of *A. nidulans alcA* promoter and investigated its phenotypes under the *ypdA*-repressed condition. Downregulation of *ypdA* transcription caused sever growth inhibition. We observed a constitutive phosphorylation of HogA MAPK in *A. nidulans ypdA* delta. These results suggest that YpdA is a essential component of the upstream of *A. nidulans* HOG (AnHOG) pathway, and the growth inhibition caused by *ypdA* delta would be attributed to disorder of signalling through the AnHOG pathway.

9. Colonies from the filamentous fungus *Aspergillus niger* are highly differentiated in spite of cytoplasmic continuity. Ronald P de Vries[1], Ana M Levin[1], Ana Conesa[2], Hildegard H. Menke[3], Manuel Talon[2], Noel NME van Peij[3], Han AB Wösten[1] [1]Microbiology, Institute of Biomembranes, Utrecht University, Padulaan 8, 3584 CH Utrecht, The Netherlands; [2]Centro de Genomica, Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain; [3]DSM Food Specialties, PO Box 1, 2600 MA Delft, The Netherlands.

Filamentous fungi play an important role in element cycling by degrading organic material. The unexplored substrate at the periphery of the fungal mycelium and the partially degraded substrate in the mycelial centre are expected to have a major impact on local gene expression. However, the continuity of the cytoplasm in a fungal mycelium and the phenomenon of cytoplasmic streaming raise the question whether there are indeed spatial differences in the mRNA composition and physiology. This was studied in 7-day-old maltose and xylose grown colonies of *Aspergillus niger*. Differential changes were detected with respect to growth, protein secretion and gene expression. Differential gene expression was not limited to specific functional categories, but occurred genome-wide. However, some gene groups related to nutrient utilization displayed significantly increased levels of differential gene expression. These data will be presented as well as evidence for the involvement of transcriptional regulators that effect the expression of specific gene groups.

10. The gene for the ubiquitin ligase regulator Cand1 is putatively split into two genes in the filamentous fungus *Aspergillus nidulans*. Elke U. Schwier, Martin Christmann, Krystyna Nahlik, Silke Busch and Gerhard H. Braus. Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Grisebachstrasse 8, 37077 Göttingen, Germany; e-mail: eschwie@gwdg.de

Ubiquitin dependent proteolysis plays an important role in many cellular processes in eukaryotes. Cullin containing ubiquitin ligases like the SCF (Skp1-Cullin-F-box protein) complex mark proteins for degradation by ubiquitinylation. It has been shown that the protein Cand1 binds to cullins. The Cand1 C-terminus blocks the SKP1 binding site of the SCF complex component Cul1 and affects thereby the assembly/disassembly of the ubiquitin ligase. The N-terminus of Cand1 buries the neddylation site on Cul1, which prevents its modification by Nedd8, an ubiquitin-like protein and alters the activity of the complex. In *A. nidulans* the gene encoding the putative homolog of human Cand1 seems to be split in two, both independent genes having about 20% identity to the human protein. Deletion of the *A. nidulans* gene coding for the protein Cand1_C similar to the c-terminal part of human Cand1 leads to a red hyphae phenotype. Cand1_C is expressed during vegetative growth but not during sexual development. It localizes to the nucleus and interacts with CulA, CulD and Cand1_N in the yeast two hybrid system. Currently we are investigating the function of the smaller protein Cand_N. The split *cand1* gene makes the fungus *A. nidulans* an attractive model organism for studying the putative different functions of the two parts of the Cand1 protein.

11. The *Aspergillus nidulans* nuclear pore complex protein An-Nup-2 plays a novel role in mitosis but is not essential for nuclear transport. Sarine Markossian and Stephen A. Osmani. The Ohio State University, Columbus, Ohio, USA. markossian.1@osu.edu

The nuclear pore complex (NPC) regulates nuclear trafficking and is composed of ~30 subunits called nucleoporins (Nups). In yeast, Nup2p has been shown to facilitate nuclear transport. Unlike yeast Nup2p, *Aspergillus nidulans* Nup2 (An-Nup2) localizes to chromatin during mitosis but to the NPC during interphase. This indicates An-Nup2 may play a role during mitosis. An-*nup2* is essential and its deletion causes mitotic defects. We therefore speculate that the localization of An-Nup2 to mitotic chromatin is important for mitosis. To test this hypothesis, a domain study was performed to define the An-Nup2 domain responsible for its mitotic translocation to chromatin and an antibody was generated against An-Nup2. A domain spanning from aa 400 to aa 1200, which encompasses a basic stretch of amino acids, a coiled coil region, and two potential nuclear localization sequences (NLS), is sufficient to locate An-Nup2-GFP to the NPC during interphase and to DNA during mitosis. The An-Nup2 antibody was used for immunofluorescence and successfully stained the nuclear periphery during interphase and chromatin during mitosis confirming the An-Nup2-GFP localization. Most importantly, the heterokaryon rescue technique was used to define if nuclear transport and/or mitosis is defective without An-Nup2. An-Nup2 deleted cells were not deficient in nuclear transport of NLS-dsRed suggesting that the lethality caused by An-Nup2 deletion is due to mitotic defects and not nuclear transport defects. In conclusion, there is emerging evidence that the localization of An-Nup2 to mitotic chromatin is essential for mitosis although An-Nup2 may not be essential for nuclear transport.

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12. A Cytoplasmic Remnant is involved during the Segregation of Nucleolar proteins in *Aspergillus nidulans* . Leena Ukil, Colin De-Souza, Hui-lin Liu, Stephen A Osmani, Department of Molecular Genetics, The Ohio State University, 484 W 12th Ave, Columbus, OH 43210, Email: leenaukil@yahoo.com

The nucleolus is a prominent nuclear structure whose mitotic segregation is poorly understood. During yeast mitosis the nucleolus segregates intact with rDNA. In contrast, during open mitosis the nucleolus is disassembled then reassembled. *Aspergillus nidulans* nuclei undergoes partially open mitosis, which is an evolutionary intermediate between open and closed mitosis. We therefore determined how *A. nidulans* nucleoli are segregated during mitosis. Unlike *Saccharomyces cerevisiae* , few *A. nidulans* nucleolar proteins segregate with DNA. Instead we have defined two patterns by which different nucleolar proteins segregate during mitosis: (1) Dispersal into the cytoplasm at the onset of mitosis but with some protein remaining bound to DNA, (2) A novel pattern in which nucleolar proteins remain in a nuclear remnant, distinct from daughter nuclei, before re-accumulating into daughter nucleoli during G1. Dual labeling of nucleolar proteins and nuclear envelope markers reveal that the nucleolar remnant is generated as a result of a double nuclear envelope fission event. This double fission occurs around a nucleolar protein mass during telophase. This mechanism generates two transport competent daughter nuclei and a very transient nucleolar remnant containing class 2 nucleolar proteins. This study indicates *A. nidulans* undergoes mitotic disassembly then reassembly of its nucleolus, as do higher eukaryotes, and that generation of daughter nuclei occurs via a double fission mechanism, not a single fission as occurs in yeasts. We suggest the novel mitotic nuclear remnant we have defined serves as a storage pool from which equal distribution of nucleolar proteins occur. It may also serve as a sink for unwanted cytoplasmic proteins or RNAs that gain access to nuclei during mitosis and may be a positional cue for the double fission.

13. The NADH oxidase *nadA* and its involvement in oxidative stress in *Aspergillus flavus*. Carrie A. Smith[1], Massimo Reverberi[2], Niki Robertson[1], Gary A. Payne[1] . [1]North Carolina State University, Raleigh, NC, USA, 27606. [2]Università "La Sapienza," Rome, Italy 00165.

nadA, which encodes a predicted NADH oxidase, was identified as part of a sugar utilization cluster that lies adjacent to the aflatoxin biosynthetic cluster in several species of *Aspergillus*. NADH oxidases convert NADH to NAD+, which is a possible coenzyme needed for reactions in the aflatoxin biochemical pathway. In a microarray experiment comparing gene expression between a wild type strain of *A. parasiticus* and a deletion mutant for the pathway regulatory gene *aflR*, *nadA* expression was significantly decreased in the mutant background. Although *nadA* is transcriptionally controlled by AflR, aflatoxin levels were unaffected in *A. flavus nadA* deletion strains under several conditions. NADH oxidases can also be a source of reactive oxygen species formation. Previous reports have shown a relationship between oxidative stress and aflatoxin production in *Aspergillus sp*. The activity of several antioxidant enzymes were examined in *nadA* deletion strains and peak activity was delayed when compared to wild type. Lower levels of lipoperoxide accumulation were also seen in *nadA* deletion strains. These data suggest that *nadA* plays a role in the oxidative stress response in *A. flavus*. Investigations are underway to characterize additional phenotypes of *nadA* deletion mutants.

14. Short term growth rate of *Aspergillus nidulans* hyphae is independent of near-apical cytoplasmic microtubule abundance. Michelle Hubbard, Susan Kaminskyj [1]. [1] Univ Saskatchewan, Saskatoon, SK S7N 5E2, Canada.

Apart from mitosis and nuclear migration, role(s) for the microtubule (MT) cytoskeleton in fungal growth rate vary between reports, likely due to strain and/or experimental differences. Using confocal microscopy, we quantified near- apical cytoplasmic MTs and hyphal growth rates in *Aspergillus nidulans* hyphae with constitutively expressed GFP-alpha-tubulin (a gift of the Oakley lab). For 219 untreated hyphae, all of which were morphologically similar but only about half of which were later found to be growing, we found no correlation between MT abundance and growth rate in the previous 2-5 min. Hyphae were treated for 30-120 min with taxol, benomyl, or latrunculin B, or with solvent controls. Considering only growing hyphae, and compared to their respective controls, MT numbers were significantly increased by DMSO, significantly reduced by benomyl, and moderately increased by latrunculin, but were unaffected by ethanol or taxol. In the same cells, growth rates were significantly increased by ethanol and taxol, unaffected by benomyl and DMSO, and reduced by latrunculin. Average hyphal growth rate in the first 30-120 min following benomyl treatment was similar to control cells, despite the absence of visible MTs after 2 min. Thus, unlike actin, we have not found a role for MTs in determining tip growth rate between 30-120 min after anti-MT treatments. Unexpectedly, growth rates of taxol-treated hyphae decreased significantly following observation by fluorescence microscopy.

15. The *Aspergillus nidulans* F-box Protein Project. Özgür Bayram[1], Heike S. Rupprecht[1], Marc Dumkow[1], Marcia R. v. Z. Kress[1], Thomas Linger[2], Özlem Sarikaya Bayram[1], Gustavo H. Goldman[3], Gerhard H. Braus[1]. [1]Institute of Microbiology & Genetics, Georg-August-University Goettingen, Grisebachstr. 8, 37077 Goettingen, Germany; e-mail: obayram@gwdg.de; hruppre@gwdg.de. [2]Institute of Microbiology & Genetics, Dept. of Bioinformatics, Georg-August-University Goettingen, Goldschmidtstr. 1, 37077 Goettingen, Germany. [3]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, CEP 14040-903, Ribeirão Preto, São Paulo, Brazil

F-box proteins are part of E3 SCF (Skp1,Cullin,F-box) ubiquitin ligases which are the specificity factors of the ubiquitin dependent protein degradation machinery. They contain the F-box as conserved N-terminal domain which acts as interface to the Skp1 protein. In addition they have a substrate binding domain for the protein to be ubiquitinated. There are at least 70 F-box-like proteins encoded by the *Aspergillus nidulans* genome. To address the cellular functions of F-box proteins in *A. nidulans*, we have started to systematically disrupt F-box encoding genes via homologous gene replacement method. The characterization of the F-box mutants, which has been carried out so far, will be presented.

16. Expression and transposition of DNA transposon *Crawler* in *Aspergillus oryzae*. Hironobu Ogasawara[1], Hiroshi Obata[2], Yoji Hata[2], Saori Takahashi[1], and Katsuya Gomi[3]. [1]Akita Research Institute for Food and Brewing, Akita, Japan. [2]Research Institute, Gekkeikan Sake Co. Ltd., Kyoto, Japan. [3]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan.

An active DNA transposon *Crawler* has been isolated and characterized from the industrially important fungus *Aspergillus oryzae*. The transposition events of *Crawler* were induced by various stress treatments such as $CuSO_4$ or heat shock. The existence of two or more transcripts in different size of *Crawler* was shown under standard culture conditions. In the present study, we analyzed the transcripts of different size by 3'- RACE analysis. Moreover, relationship between the transposition activity and the proportions of *Crawler* mRNA molecules was also studied to clarify the control mechanism for transcription of exogenous gene *Crawler*. The smaller transcribed fragments were resulted from premature polyadenylation and in some cases erroneous intron splicing within the transposase. The erroneous splicing tends to be inhibited by stress treatment of $CuSO_4$, which stimulated the transposition events in conidia allowing the full-length and active transposase to be produced. These results indicate that *A. oryzae* has a defense system against the exogenous active genes like transposons by mRNA quality control system such as undesirable splicing or polyadenylation resulted in nonstop mRNA decay.

17. The *Aspergillus nidulans snxA1* and *nimA5* mutations interact to affect mitotic spindle structure. Yulon Stewart, Ryan Day, Kirk Jackson, Michael Jackson, and Sarah Lea McGuire, Millsaps College, 1701 N. State St., Jackson, MS, 39210.

Both the *nimA* and *snxA* genes interact with $nimX^{cdc2}$ to affect mitosis in *Aspergillus nidulans*. *nimA* affects the nuclear import of $nimX^{cdc2}$, while the *snxA1* mutation is a cold-sensitive suppressor of the $nimX2^{cdc2}$ mutation. *snxA1* leads to abnormal nuclear morphology at 17°C. To better understand effects of the *snxA1* mutation on cells and the relationship between *snxA* and *nimA*, we generated strains expressing GFP- *tubA* (alpha-tubulin) and various combinations of *snxA* and *nimA* mutations. At 17°C *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 others were bifurcated or trifurcated and had fragmented, variably condensed nuclei along their lengths. Similar abnormal nuclei and spindle structures were observed when *snxA1/nimA1/*GFP-*tubA* cells and *snxA1/nimA5/*GFP-*tubA* cells were germinated at 32°C and upshifted to 44°C for 3 hours, suggesting that the effects of *snxA1* on *nimA* are not allele-specific. After 3 hours at 44°C, 69% of *snxA1/nimA1/*GFP-*tubA* cells had abnormal nuclei, and 56% had abnormal spindles; similar results were obtained with *snxA1/nimA5/*GFP-*tubA* cells. Confocal microscopy of the abnormal spindles shows highly unusual spindle structures, which are more severe in cells carrying the *snxA1/nimA5* double mutant; *snxA1/nimA1* double mutant cells often have significantly shortened spindles. Efforts to clone the *snxA* gene are ongoing and should aid in the understanding of the interactions of the *snxA* and *nimA* genes in mitotic control. Supported by NIH R15GM55885 and NIH RR016476 from the MFGN INBRE program of the NCRR.

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18. Role of vesicular trafficking in nutrient sensing. Margaret E. Katz[1], Cara J. Evans[1], Joan M. Kelly[2] and Brian F. Cheetham[1], [1]University of New England, Armidale, NSW Australia and [2]University of Adelaide, SA, Australia. mkatz@une.edu.au

The *xprG* gene encodes a putative transcriptional activator involved in the response to nutrient limitation. Strains carrying an *xprG*$^-$ null mutation do not produce extracellular proteases in response to carbon limitation. We have isolated revertants of an *xprG*$^-$ mutant. The revertants carry mutations in genes which we have named suppressors of *xprG* (*sogA*, *sogB* and *sogC*). Two of the revertants carry chromosome rearrangements. A combination of genetic mapping and Southern blot analysis was used to show that the translocation breakpoint in the *sogA1* mutant was located in a gene encoding a sorting nexin (Vps5). Transformation with a wild type copy of the gene confirmed that the *sogA* gene encodes Vps5. Mapping data indicated that *sogB* was tightly linked to a gene on chromosome VII encoding another component of the multivesicular body pathway, Vps17 and complementation of *sogB1* with the gene encoding the *A. nidulans* Vps17 homolog confirmed the identification. Vps17 and Vps 5 are part of a complex that has been shown to be involved in sorting of vacuolar proteins (*e.g.* intracellular proteases) in yeast and regulation of cell-surface receptors in mammals. Two hypotheses could account for the ability of mutations in *sogA* and *sogB* to suppress loss-of-function mutations in *xprG*. Mutations in *sogA* and *sogB* may permit secretion of intracellular proteases (as in *S. cerevisiae*) or an alteration in cell-surface receptors may trigger an XprG-independent mechanism for activation of extracellular protease gene expression.

19. A RasGAP protein involved in polarity establishment and maintenance in *Aspergillus nidulans.* Laura Harispe[1,2,3], Lisette Gorfinkiel[2], Cecilia Portela[2], Miguel A. Peñalva[3], and Claudio Scazzocchio[1]. [1]IGM, Univ. Paris-Sud XI. Bat 409 Centre d´Orsay. 91405 Orsay(France). [2]F. Ciencias, Univ. de la República. Iguá 4525. 11400 Montevideo(Uruguay) [3]CIB, CSIC, Ramiro de Maeztu 9. 28040 Madrid(Spain)

Filamentous fungi represent an extreme example of polarised growth[(a)]. We report on the identification and characterisation of GapA, an *A. nidulans* RasGAP involved in polarity establishment and maintenance. GapA was identified after serendipitously isolating a partial loss-of-function mutation, designated *gapA1*, in a genetic screen. Phenotypic characteristics resulting from *gapA* deletion include compact colony morphology, a marked delay in polarity establishment during conidial germination, impairment of polarised hyphal extension, a conspicuous developmental defect typically manifested by the absence of one layer of sterigmata in the conidiophore and a defect in the otherwise polarised distribution of the actin cystoskeleton. GapA-GFP protein fusion expressed from a gene replacement allel appears localises to hyphal tips and septa. This localisation suggests that a Ras protein(s), whose activity is antagonised by GapA plays a role in the regulation of the actin cytoskeleton at the hyphal tip and that this abnormal regulation underlies the polarity phenotypes associated with *gapA* loss-of-function.

[(a)] Momany M. (2002),Curr. Opinion in Microbiology, 5:580–585.

20. A link between N-myristoylation and proteasome activity. Soo Chan Lee and Brian D. Shaw Program for the Biology of Filamentous Fungi/Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, 77840

Ubiquitin dependent proteolysis is a fundamental biological process regulating the half-life of proteins. The interaction between the 19S and 20S proteasome particles is essential for this activity. A subunit of the 19S particle, RptA in *A. nidulans*, has a conserved N-myristoylation motif. Orthologs of RptA are known to control substrate entry and gate the channel of 20S particle, but less is known about the role of N-myristoylation of the protein on its function. In our analyses of genetic suppressors of an A. nidulans N-myristoyl transferase mutant (*swoF1*), we found a mutation in a 20S proteasome alpha subunit that partially bypasses phenotypic defects of the *swoF1* mutant. To investigate the mode of suppression, we used monoclonal anti-ubiquitin antibody to measure the amount of the ubiquitinated proteins. The *swoF1* mutant accumulates fewer ubiquitinated proteins than does wild-type. The suppressor mutant, however, accumulated more. In addition, the abnormal hyphal growth phenotypes of the *swoF1* mutant were partially bypassed in the presence of MG132, a proteasome inhibitor. These data suggest that N-myristoylation negatively regulates the proteasome activity. We will discuss our ongoing investigation of the role of myristoylation of RptA protein on i) function of the proteasome, ii) the interaction between 19S and 20S proteasome and iii) the localization of 19S proteasome.

21. The role of ADP-ribosylation factors in cell morphogenesis of *Aspergillus nidulans.* Soo Chan Lee and Brian D. Shaw Program for the Biology of Filamentous Fungi/Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, 77840

ADP-ribosylation factors (ARFs) are small GTPase proteins with several biological activities including vesicle formation and trafficking and, in yeast, bud site selection. In filamentous fungi, numerous vesicles are found at the growing tips and in the Spitzenkorper where they are thought to be active in secretion of cellular components, endocytosis, and maintenance of tip growth. The exact roles of the ARFs in filamentous fungi have not been established. ArfA::GFP localizes to cellular compartment which may be Golgi. ArfA::GFP was not co-localized with endocytosis machineries indicating ArfA is involved in exocytosis. ArfB::GFP localized to septa, a new cell wall synthesis site. Disruption of ArfB by transposon insertion resulted in loss of polarity during germ tube emergence and hyphal growth. A compromised Spitzenkorper was observed in the mutant. In addition, the arfB::Tn strain displayed delayed endocytosis. The Arf proteins have a conserved N-myristoylation motif. In *swoF1* (N-myristoyl transferase) mutant cells, ArfA::GFP and ArfB::GFP showed non-specific localization. In wild type cells, $ArfA^{G2A}$::GFP and $ArfB^{G2A}$::GFP, each with a G2A amino acid substitution that disrupts myristoylation, mislocalized. Interestingly overexpression of ArfA protein partially rescues the polarity defect of the *swoF1* mutant. These observations suggest that both endocytosis and exocytosis by the Arf proteins play a critical role in hyphal polarized growth in filamentous fungi and N-myristoylation determines subcellular localizations for ArfA and ArfB.

22. A putative MAP-kinase, MpkB, regulates natural product biosynthesis in *Aspergillus nidulans.* Dapeng Bao and Ana M. Calvo Department of Biological Sciences, Northern Illinois University, 1425 W Lincoln Hwy., Dekalb, Illinois. 60115, USA

Mitogen-activated protein kinase pathways transduce a large variety of external signals. This essential funtion is conserved in many eukaryotes, including fungi. In this work we studied the role of *mpkB*, encoding a putative homolog of the *Saccharomyces cerevisiae* FUS3 MAP-kinase, on the biosynthesis of natural products in the model filamentous fungus *Aspergillus nidulans*. We found that the *mpkB* loss-of-function mutant not only presented reduced production of the mycotoxin sterigmatocystin but also showed alterations in the biosynthesis of other natural products. The *mpkB* mutant only produced trace amounts of penicillin under conditions that promoted the production of this antibiotic in the wild type (approximately 27-fold greater). Furthermore, we found that expression of *ipnA*, encoding an isopenicillin synthase, is substantially reduced in the *mpkB* mutant. The complemented strain restored sterigmatocystin and penicillin wild-type levels. These results suggest that the MAPK signaling cascade regulates secondary metabolism in an adaptive response to environmental stimuli.

23. Effect of osmotic stress on sclerotial and aflatoxin production in *Aspergillus flavus*. Rocio M Duran and Ana M Calvo Department of Biological Sciences, Northern Illinois University, 1425 W Lincoln Hwy, DeKalb, Illinois.

Fungal cells respond to osmotic stress with molecular adaptations that result in chemical and morphological changes. We examined the effect of environmental osmotic stress on the fungus *Aspergillus flavus*. We found that hypertonic media differentially affected the formation of resistant structures, sclerotia, and the biosynthesis of the carcinogenic mycotoxin aflatoxin. While aflatoxin biosynthesis slightly increased in media with high concentration of sodium chloride or sorbitol, production of sclerotia was greatly reduced. The strong reduction of sclerotia was accompanied by an increase in colony growth and conidiophore formation. Search of the *A. flavus* genomic database allowed us to identify *AFhogA*, a gene encoding a putative homolog of the mitogen-activated protein kinase HOG1 that mediates the osmotic stress response in *Saccharomyces cerevisiae*. Additional components of this signaling pathway were also found in the *A. flavus* genomic sequence. We are further investigating the implications of this pathway in the regulation of resistant structure formation in this important plant pathogen.

24. A CLASP-related protein acts at the spindle pole body and spindle midzone during mitosis in *Aspergillus nidulans*. C. Tracy Zeng, and Bo Liu. Section of Plant Biology, University of California, Davis, CA 95616, USA.

In *A. nidulans*, microtubules undergo rapid reorganization during mitosis inside growing hyphae. Proteins like CLASP (CLIP170-associated protein) are known to play critical roles in regulating microtubule dynamics in mitotic spindles in animal cells. CLASP, also known as Orbit/Mast, is among a class of microtubule plus end-tracking proteins or +TIPs, and plays a critical role in attaching the kinetochore to microtubules during mitosis. In the *A. nidulans* genome, the AN0995 locus codes a polypeptide with limited sequence homology with the mammalian CLASP. In order to learn the function of this CLAA, for CLASP-like protein A, we attempted to knock out the *claA* gene by homologous recombination. Because none of over 100 transformants was viable, the result indicated that *claA* could be an essential gene. To further elucidate the function of CLAA, a strain was created in which a CLAA-GFP fusion protein was expressed under the control of the native promoter. CLAA-GFP only exhibited distinct localization pattern during mitosis. It first appeared at the spindle pole body. In addition to decorating the spindle pole body, at anaphase CLAA-GFP was associated with spindle midzone. In contrast to CLASP localization in animal cells, CLAA did not act as a typical +TIP in *A. nidulans*. Results of testing interactions between CLAA and other microtubule-associated proteins will be presented.

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25. The roles of fimbrin, *fimA*, and alpha-actinin, *acnA*, in hyphal growth. Srijana Upadhyay[1], Aleksandra Virag[2], Soo Chan Lee[1], Steven D. Harris[2], and Brian D. Shaw[1]. [1]Dept of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77803, USA. [2]Plant Science Initiative, University of Nebraska-Lincoln, N234 Beadle Center, 1901 Vine Street, Lincoln, NE 68588, USA.

We investigated the roles of actin (ActA) binding proteins fimbrin (FimA) and alpha-actinin (AcnA) in hyphal growth in *A. nidulans*. We have used live cell imaging to examine the distribution of ActA::GFP, FimA::GFP and AcnA::GFP. In actively growing hyphae cortical ActA::GFP and FimA::GFP patches are highly mobile and are concentrated near the hyphal apex, but a patch depeleted zone occupies the hyphal apex. FimA::GFP localizes transiently to septa. Localization of both ActA::GFP and FimA::GFP was disrupted after cytochalasin treatment. AcnA::GFP localizes to septal rings and has not been visualized at hyphal apices. A transposon insertional strategy was used to disrupt *fimA* resulting in germinating conidia with an extended isotropic growth phase followed by simultaneous emergence of multiple germ tubes. Colonies of the *fimA* disruptants are compact and conidiate poorly. Deletion of *acnA*, results in a severe hyphal growth defect leading to compact colonies that not sporulate. Endocytosis was severly impaired in the *fimA* disruption strain but was unaffected in the *acnA* deletion strain. ActA::GFP distribution in the *fimA* disruption strain, results in abnormal ActA::GFP distribution. A model for the roles of these proteins in hyphal growth is proposed.

26. A mutation in a GDP mannose pyrophosphorylase encoding gene leads to aberrant hyphal growth. *Brian D. Shaw, Gustavo Rebello, Soo Chan Lee, Srijana Upadhyay and Melissa Long Program for the Biology of Filamentous Fungi, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77803, USA.

The temperature sensitive *swoN1* mutant results in an aberrant hyphal growth pattern that differs significantly from wild type. Growth from conidia results in germlings with pronounced swollen sub-apical cell compartments as much as 20 micron in diameter. Growth and development at hyphal apices appears to proceed similarly to wild type. The *swoN1* mutant was complemented using a genomic library, in which two genes were identified with potential to restore growth of the mutant to wild type levels. These genes are AN1911.3 and AN5586.3 as designated by the Broad Institute *A. nidulans* genomic database; each encode proteins with predicted similarity to GDP mannose pyrophosphorylase. We designate AN1911.3 *swoN* since it contains a point mutation at position 1240 bp after start of genomic sequence and 1093 bp after the start codon of coding sequence. This point mutation results in a predicted residue change of serine to a phenylalanine at amino acid 365 of the protein. The *swoN1* mutant also exhibits an altered staining pattern of the mannoprotein stain Alcian Blue, relative to wild type. This gene along with *swoM*, *manA* and *swoA* represents the fourth identified in *A. nidulans* that is likely to participate in protein mannosylation. A model for the role of protein mannosylation in hyphal growth is discussed.

27. The *Aspergillus nidulans snoA* inhibitor of cell division associates with the BRDF checkpoint domain of *nimO*^{Dbf4}. Steve James, James Barra, Megan Campbell, and Matthew Denholtz. Biology Department, Gettysburg College, Gettysburg, PA. sjames@gettysburg.edu

In *Aspergillus nidulans*, *nimO*^{Dbf4} and *cdc7* encode regulatory and catalytic subunits of the conserved DBF4-dependent kinase (DDK). DDK initiates DNA synthesis by phosphorylating the replicative DNA helicase to trigger DNA unwinding at origins of replication. In addition, DBF4 plays an important role in the DNA damage response. This role is mediated by an N-terminal BRDF motif (*BR*CT and *D*B*F*4 similarity domain), as revealed by mutations in yeast homologs that confer enhanced sensitivity to DNA damage agents and failure to restrain DNA synthesis during genotoxic stress. In *Saccharomyces cerevisiae*, *RAD53/CHK2* kinase is the only checkpoint mediator known to associate directly with the *DBF4* BRDF motif. We identified a novel inhibitor of *nimO*^{Dbf4} called *snoA* (*s*uppressor-of-*n*im*O*). Loss of *snoA* rescues *nimO*18 ts-lethality and hypomorphic *nimO*+ expression. Conversely, *snoA* overexpression confers a dose-dependent, lethal interphase cell cycle arrest in *nimO*18 cells. Here we report a novel interaction between the *nimO* BRDF motif and *snoA*. Using yeast two-hybrid analysis, we demonstrate that a short (~100 amino acid) serine- and proline-rich region in the *snoA* C-terminus can associate with the *nimO* BRDF. This novel discovery suggests that *snoA* may act to regulate normal DNA synthesis or to exert S phase checkpoint control by direct association with the *A. nidulans* DBF4-dependent kinase. (Supported by NSF-RUI #01-14446 to SJ)

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28. *Aspergillus oryzae atfA* encodes a transcription factor, which is required for vigorous growth in the solid-state fermentation. K. Sakamoto[1], O. Yamada[1], Y. Okita[1], K. Iwashita[1], O. Akita[2], K. Gomi[3], S. Mikami[1] 1 National Research Institute of Brewing, 3-7-1 Kagamiyama Higashi-Hiroshima, Hiroshima, Japan. 2 Jissen women's University, 4-1-1 Osakaue, Hino, Tokyo, Japan. 3 Tohoku University, 1-1 Tsutsumidori-Amamiyatyou Aoba Sendai, Miyagi, Japan

In the solid-state culture, *Aspergillus oryzae* exhibits phenotypes such as the high production of enzymes and conidiophore development. 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 a gene encoding transcription factors that show high homology to *atf1* of *Shizosaccharomyces pombe* and named it *atfA*. We constructed *atfA*-deletion strain (DelA51) to analyze the function. The germination ratio of DelA51 conidia was reduced to 13.7%, while that of wild type (wt) conidia was over 90 %. Furthermore the DelA51 conidia were more sensitive to stress than wt. Although DelA51 grew as fast as wt in the submerged liquid culture, the growth of DelA51 was attenuated in solid-state fermentation. High humidity of atmosphere restored the growth of DelA51. Thus we concluded that *atfA* is necessary for the vigorous growth on the low water activity substrate. To determine *atfA*-regulated genes, we identified 34 cDNAs down regulated in DelA51 under osmotic stress condition using mycroarray.

29. Putative mannose transporters complement a branching/septation defect in *Aspergillus nidulans*. Loretta Jackson-Hayes, Lauren Fay, Terry W. Hill and Darlene M. Loprete Departments of Biology and Chemistry, Rhodes College, Memphis, TN 38112. jacksonhayesl@rhodes.edu

In order to identify novel genes affecting cell wall integrity, we have generated mutant strains of the filamentous fungus *Aspergillus nidulans*, which show hypersensitivity to the chitin synthase inhibitor Calcofluor White (CFW). The phenotype of one of these strains (R205) also shows morphological abnormalities related to branching and septation. We have cloned two DNA fragments from an *A. nidulans* genomic DNA library which improve resistance to CFW and restore a more normal phenotype. One fragment is gene AN8848.3, “MT1”, which shows homology to GDP- mannose transporters. The second fragment is gene AN9298.3, “MT2”, which is a similar but distinct gene also homologous to GDP-mannose transporters. When separately cloned, the putative GDP-mannose transporters restore normal phenotype including full restoration of subapical hyphal compartment length and branch density in the mutant. Sequencing reveals a genetic lesion in Exon 5 of MT1 which causes an alanine to proline substitution and no mutation in MT2 in mutant strain R205. Cloned R205 MT1 containing the Exon 5 mutation does not complement the R205 phenotype. Attempts to produce null mutants of MT1 did not produce viable transformants, suggesting that AN8848.3 is an essential gene. MT2 null mutants grow normally under normal growth conditions and show wild type CFW resistance.

30. Characterization of *radC*, the *Aspergillus nidulans* homolog of *RAD52*, a key gene for DNA repair by homologous recombination. Michael Lynge Nielsen, Gaëlle Lettier, Jakob Blæsbjerg Nielsen and Uffe Hasbro Mortensen

Repair of DNA double-strand breaks (DSBs) is crucial for maintaining genome integrity and failure to repair even a single DNA DSB is lethal as it causes loss of part of a chromosome during cell division. In the yeast, *Saccharomyces cerevisiae*, Rad52 plays a fundamental role in the repair of DSBs by homologous recombination (HR) and among genes involved in DNA DSB repair and HR deletion of *RAD52* produces the most dramatic phenotype. Hence, *S. cerevisiae* cells lacking Rad52 are impaired in DSB repair and all types of HR including targeted integration of transforming DNA. Orthologs of *RAD52* have been identified in other organisms, including *Schizosaccharomyces pombe*, *Aspergillus nidulans*, chicken, mouse, and human. However, in contrast to *S. cerevisiae*, the absence of *RAD52* in higher eukaryotes does not result in a severe phenotype, due to the evolution of additional repair pathways and the role of Rad52 in DNA DSB repair and HR remains unclear. To address the role of Rad52 in higher eukaryotes, we have recently constructed a *radC* (homolog of *RAD52*) deletion mutant in *A. nidulans* and performed an initial characterization of the mutant strain. Unlike in *S. cerevisiae*, where Rad52 is essential for the repair of all types of DNA lesions that require HR, we show that in *A. nidulans* it is only required for the repair of a subset of these lesions. Hence, in an evolutionary perspective, HR repair in *A. nidulans* may represent an intermediate state between *S. cerevisiae* and human and *A. nidulans* may therefore be a useful model to further the understanding of HR in higher eukaryotes.

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COMPARATIVE AND FUNCTIONAL GENOMICS

31. Chemogenomics of antioxidant inhibition of aflatoxin biosynthesis. Jong Kim[1], Jiujiang Yu[2,3], Noreen Mahoney[1], Kathleen Chan[1], Deepak Bhatnagar[2], Thomas Cleveland[2], Russell Molyneux[1], William Nierman[3,4] and Bruce Campbell[1*]. [1]Plant Mycotoxin Research, USDA, Albany, CA 94710 USA. bcc@pw.usda.gov. [2]Food and Feed Safety Research, USDA, New Orleans, LA 70124 USA. [3]The Institute of Genomic Research, Rockville, MD 20850 USA. [4]The George Washington University, Washington, DC 20006 USA.

Natural compounds were used to probe the functional genomics (chemogenomics) of aflatoxin biosynthesis. Caffeic acid (12mM) treatment of *Aspergillus flavus* inhibited aflatoxin biosynthesis without affecting growth. Microarray analysis showed genes in the aflatoxin biosynthetic cluster were completely down-regulated (log2 ratio –0.04 to –3.13). However, aflatoxin pathway regulator genes, *aflJ* or *laeA*, and sugar utilization cluster genes showed only minor changes in expression (log2 ratio 0.08 to –0.58). Genes in amino acid biosynthetic and aromatic compound metabolism were up-regulated (log2 ratio > 1.5). Most notable was up-regulation (log2 ratio 1.08 to 2.65, qRT-PCR) of four peroxiredoxin genes orthologous to the *AHP1* gene (alkyl hydroperoxide reductase) of *Saccharomyces cerevisiae*. Antioxidants trigger induction of *ahp* genes in *A. flavus* to protect the fungus from oxidizing agents, e.g., lipoperoxides, reactive oxygen species, etc. This detoxification attenuates upstream oxidative stress-response pathway signals, thus suppressing aflatoxigenesis.

32. Transcriptional activators of *Aspergillus nidulans* sulfur metabolism. Sebastian Pilsyk, Jerzy Brzywczy and Andrzej Paszewski. Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawiñskiego 5A, 02-106 Warsaw, Poland e-mail: Seba@ibb.waw.pl

Aspergillus nidulans metR gene encodes a bZIP transcription factor specific for activation of several sulfur metabolism genes including these encoding the sulfate assimilation pathway enzymes. METR protein level and/or activity is negatively regulated by SCF ubiquitin ligase complex which consists of the proteins encoded by the *scon* (sulfur controller) genes. Both *metR* and *scon* genes constitute a regulatory system known as sulfur metabolite repression (SMR). Loss of function mutations in the *metR* gene cause methionine auxotrophy while mutations in the *scon* genes lead to a derepression of the sulfate assimilation pathway enzymes. All *metR* and *scon* mutants isolated so far were recessive. Recently, we have isolated three dominant suppressor mutants in a diploid strain homozygotic for *metB3* allele. Genetic and molecular analysis showed that Phe48 codon of the *metR* gene is affected in every of the dominant mutants obtained (*MetR20*, *21* and *22*). The orthologs of the *metR* gene were also found in other fungal species and Phe48 appears to be one of the most conserved amino acids of the N-terminal domain which suggests that this residue is important for METR protein function and/or stability. *MetR20* mutant has elevated acivity of the sulfur metabolism enzymes in comparison to the wild type. Suppressor *metR* mutants have also an increased level of sulfur compounds in mycelia which suggests that they are resistant to SMR. By searching of the *A. nidulans* genome sequence, we have found a paralog of the METR protein that encoded protein has similar basic region and leucine zipper sequences. The gene we named *metZ* is well conserved among other *Aspergilli* and it has apparently unusual large conserved intron as long as intron in the *metR* gene. Northern blot analysis indicates that the *metZ* gene is regulated by SMR.

33. Relative Protein Quantification through Stable Isotope Labeling by Amino Acids in *Aspergillus flavus*: Temperature Regulation of Aflatoxin Biosynthesis. D. Ryan Georgianna[1,3], David C. Muddiman[2], and Gary A. Payne[1]. [1]Department of Plant Pathology and Center for Integrated Fungal Research. [2]Department of Chemistry. [3]Functional Genomics Graduate Program. North Carolina State University,Raleigh, NC 27606 USA.

Aflatoxin biosynthesis is inhibited at 37C, the optimum temperature for growth of *Aspergillus flavus*. Transcriptional analysis has shown that all aflatoxin biosynthetic genes except the pathway regulatory genes *aflR* and *aflS* are down regulated at 37C relative to 28C, suggesting that AFLR may be modified at 37C. To quantify the response of AFLR and other proteins to high temperature we adapted a stable isotope labeling by amino acids (SILAC) strategy for relative protein quantification in *A. flavus*. SILAC relies on the quantitative incorporation of labeled amino acids into proteins to provide a powerful mass spectrometry based proteomics tool useful for both the rapid quantification of proteins and identification of interactions. This technique has been used in several systems; including yeast, mammalian cells, and *Arabidopsis* cell culture. Samples were prepared using in-gel trypsin digestion of selected 1D-PAGE gel slices and analyzed on an ESI LTQ linear ion trap mass- spectrometer directly coupled to RP-HPLC. The *A. flavus* labeling strategy was optimized to provide a homogenously labeled sample with ~90% incorporation of [13C6] arginine. Furthermore, we found that the relative abundance of aflatoxin pathway enzymes compared between 28C and 37C is consistent with the relative abundance of their encoding transcripts at the respective temperatures. This is the first report of SILAC being used to quantify proteins in a filamentous fungus as well as a multi-cellular free-living prototrophic organism.

34. Comparative and functional genomics in identifying aflatoxin biosynthetic genes. Jiujiang Yu[1,7], Jeffery Wilkinson[2], Gary Payne[3], Masayuki Machida[4], Bruce Campbell[5], Joan Bennett[6], Deepak Bhatnagar[1], Thomas Cleveland[1], and William Nierman[7,8]. [1]USDA/ARS, Southern Regional Research Center, New Orleans, LA 70124, USA; [2]Mississippi State University, Mississippi State, MS 39762, USA; [3]North Carolina State University, Raleigh, NC 27695, USA; [4]National Institute of Advanced Industrial Science and Technologies (AIST), Tsukuba, Ibaraki, Japan; [5]USDA/ARS, Western Regional Research Center, Albany, CA 94710, USA; [6]Rutgers University, New Brunswick, NJ 08901, USA; [7]The Institute for Genomic Research, Rockville, MD 20850, USA; [8]The George Washington University School of Medicine, Washington, DC 20037, USA

Identification of genes involved in aflatoxin biosynthesis through *Aspergillus flavus* genomics has been actively pursued. *A. flavus* Expressed Sequence Tags (EST) and whole genome sequencing have been completed. Groups of genes that are potentially involved in aflatoxin production have been profiled using microarrays under different culture conditions and during fungal infection of corn. Preliminary annotation of the sequence revealed that there are about 12,000 genes in the *A. flavus* genome. Many genes in the genome, which potentially encode for enzymes involved in secondary metabolite production, such as polyketide synthases, non-ribosomal peptide synthases, cytochrome P450 monooxigenases, have been identified. Comparative analysis of *A. flavus* genome with food grade industrial fermentation organism *A. oryzae* can help understanding the mechanism of aflatoxin biosynthesis and solving the problem of aflatoxin contamination.

35. Growth and developmental control in *Aspergillus nidulans* and *A. fumigatus*. Jae-Hyung Mah and Jae-Hyuk Yu Department of Bacteriology, University of Wisconsin, Madison, WI 53706

The opportunistic human pathogen *Aspergillus fumigatus* reproduces by forming a large number of asexual spores. We studied the mechanisms regulating asexual development in *A. fumigatus* via examining functions of four key controllers, GpaA (G alpha), AfFlbA (RGS), AfFluG and AfBrlA. Expression analyses of gpaA, AfflbA, AffluG, AfbrlA and AfwetA revealed that, whereas transcripts of AfflbA and AffluG accumulate constantly, AfbrlA and AfwetA are specifically expressed during conidiation. Both loss of function AfflbA and dominant activating GpaAQ204L mutations resulted in reduced conidiation coupled with increased hyphal mass, indicating that GpaA mediates signaling that activates vegetative growth while inhibiting conidiation. As GpaA is the principal target for AfFlbA, the dominant interfering GpaAG203R mutation suppressed the phenotype resulting from loss of AfflbA function. These results corroborate the idea that parimary roles of G proteins and RGSs are conserved in aspergilli. Functions of the two major developmental activators AfFluG and AfBrlA are then examined. While deletion of AfbrlA eliminated conidiation completely, deletion of AffluG did not cause severe defects in *A. fumigatus* sporulation in air-exposed culture, implying that, whereas the two Aspergillus species may have a common key downstream developmental activator, upstream mechanisms activating brlA may be distinct. Finally, both AffluG and AfflbA mutants showed reduced conidiation and delayed accumulation of AfbrlA mRNA in developmental induction, indicating that these upstream regulators are associated with the proper progression of conidiation.

36. Integrated database for functional analysis in *Aspergillus flavus*.C. P. Smith[1], C. P. Woloshuk[2], N. P. Keller[3], J. Yu[4], and G. A. Payne[5] [1]North Carolina State University, Raleigh, USA. chris@statgen.ncsu.edu. [2] Purdue University, West Lafayette, IN, USA. woloshuk@purdue.edu. [3]University of Wisconsin, Madison, WI, USA. npk@plantpath.wisc.edu. [4]USDA/ARS/SRRC, New Orleans, LA, USA. jiuyu@srrc.ars.usda.gov. [5]North Carolina State University, Raleigh, USA. gary_payne@ncsu.edu.

Aspergillus flavus is a plant and animal pathogen that also produces the carcinogen, aflatoxin. Because of its economic importance and well characterized pathway of aflatoxin biosynthesis, several labs are studying the development, metabolism, ecology, and pathogenicity of this fungus. To facilitate the research efforts in these areas and to identify potential genes and pathways for functional analysis, we are developing a database to integrate multiple categories of data. This database resource will serve two important functions: 1) it will provide a platform for the deposition of data from individual experiments; and 2) it will permit the ready analysis of composite data from all experiments enabling researchers to mine a larger data set. It will include phenotypic measurements, gene expression data from microarrays, and metabolic profile information and will be flexible enough to allow the addition of new types of measurement in the future. Users will interact with the database through a web based interface and will be able to: describe experiments; upload data gathered during those experiments; run analyses on the data; select and download raw data; select and download the results of analyses. Recently acquired lab and field data will be used to highlight the structure and utility of this database. These measurements will include fungal growth, aflatoxin concentrations, and gene expression data acquired from lab studies and infected developing maize seeds. This research was funded by USDA/NRI/CGP 2006-35604-16666.

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37. Aspergillus Comparative Database: a web-based tool for comparative analysis. Vinita Joardar, Jonathan Crabtree, Rama Maiti, Natalie Fedorova, Paolo Amedeo, Samuel Angiuoli, William Nierman, Owen R. White, and Jennifer R. Wortman. The Institute for Genomic Research, Rockville, MD, USA. vinita@tigr.org

Comparative genome analysis in the genus Aspergillus has been facilitated by the availability of genome sequences for multiple species. Ortholog clusters were computed based on the mutual best blastP hits between the Aspergillus proteomes. Syntenic blocks were identified by searching for collinear orthologs, with allowances for gaps and rearrangements, along the full chromosomes and/or supercontigs. The results of the genome and proteome level computes for the Aspergillus genomes were stored in the Aspergillus Comparative Database (asp), a chado relational database. Sybil, a web-based software package developed at TIGR, was used for visualization and analysis of comparative genomics data. Sybil uses a graphical user interface to present and navigate the information stored in asp. The interactive graphical displays allow the user to navigate from global genome views down to specific protein reports. Protein cluster reports, lists of singletons, comparative sequence displays and publication-quality figures can be customized based on user specifications. The Sybil package also leverages the comparative data for annotation improvement. We present an overview of the Sybil package applied to the comparative analysis of aspergilli (http://www.tigr.org/sybil/asp/index.html). The Sybil visualization software is freely available for download from http://sybil.sf.net/

38. A comparison of the expression of secreted hydrolases in *Aspergillus fumigatus* and *Aspergillus nidulans* under phospholipid rich conditions. Manda Gent, Karin Lanthaler, David Denning, Michael Wilson, Stephen Oliver and Geoffrey Robson. Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK.

Aspergillus fumigatus is an opportunistic pathogen of man and is a particular risk for immunocompromised patients. Considerable research is being directed to the identification of pathogenicity determinants which may explain the virulence of *A. fumigatus* over other common environmental fungal species. Extracellular phospholipases have been shown to be important virulence determinants in the pathogenesis of several bacterial infections where they cause tissue damage and necrosis. It was recently demonstrated that clinical isolates of *A. fumigatus* produce significantly higher levels of extracellular phospholipase C activity compared with environmental isolates [Birch *et al*., 2004]. This may be important in disease development, as infection usually occurs through the inhalation of airborne conidia and the surface of the alveoli is coated in surfactant which is composed of >80% phospholipid. We have examined gene regulation in the presence and absence of lecithin, a phosphatidylcholine phospholipid, similar to that found in the lung, using the TIGR microarrays of the *A. fumigatus* and *A. nidulans* genomes. Analysis showed significant differential regulation of a number of secreted lipases as well as known allergens and virulence-related genes in *A. fumigatus* and the far less pathogenic *A. nidulans* under the same conditions.

39. Revealing components of the glucose sensing and repression pathways in *Aspergillus niger.* Margarita Salazar*, Michael L. Nielsen, Jens Nielsen Center for Microbial Biotechnology, BioCentrum, Technical University of Denmark, DK 2800 Kgs. Lyngby, Denmark. *masa@biocentrum.dtu.dk

Carbon repression is a global regulatory mechanism in which the presence of glucose or other readily metabolized carbohydrates represses expression of genes involved in the utilization of less-favored carbon sources. Mig1 is the major transcription factor responsible for carbon catabolite repression in *Saccharomyces cerevisiae* and its homologue, CreA is present in several aspergilli. Nevertheless, no mechanisms have been identified by which CreA is regulated, as well as no components for glucose sensing have been recognized. Comparative analysis of proteins involved in glucose sensing and repression pathways in *S. cerevisiae*, *A. niger*, *A. nidulans* and *A. oryzae* was performed. The analysis showed that some proteins involved in carbon catabolite repression in yeast may also play a role in aspergilli. The sensors Snf3 and Rgt2 from *S. cerevisiae* seem to be present in *A. niger*, as well as homologues to the regulatory protein Grr1 (e-value = $3E^{-92}$, score = 335) and the protein phosphatase Glc7 (e-value = $1E^{-149}$, score = 523) involved in regulating the activity of the Snf1-complex. A disrupted *grr1* mutant was constructed through the bipartite gene targeting method. The deduced amino acid sequence from the *grr1* locus showed 59 % identity to the *Aspergillus nidulans* FGSC A4 gene product (1576 aa). According to the Joint Genome Institute, the coding sequence is 4.8 kb long with the capacity to express a 1615 aa protein interrupted by five introns. Characterization of the *grr1* mutant is being performed and the morphology compared to the wild type was different during growth on several media plates. This study will be complemented by transcriptome analysis.
The authors acknowledge Conacyt and SEP Mexico for providing research fellowship to Margarita Salazar.

40. Dual genome microarray: *Fusarium verticillioides* and *Aspergillus flavus* gene expression in co-culture. Daren W. Brown Mycotoxin Research Unit, U.S. Department of Agriculture-ARS, Peoria, IL 61604

Aflatoxins produced by *Aspergillus flavus* and fumonisins produced by *Fusarium verticillioides* are prominent among the mycotoxins associated with economic losses to the maize grain industry worldwide. *F. verticillioides* is also recognized as a systemic endophyte of maize that prevents opportunistic saprotrophs such as *A. flavus* from spreading within the ear and rotting the seeds. Protective endophtyes are potential sources of gene products that can suppress fungal growth or silence genes critical to mycotoxin synthesis that are *a priori* adapted to function *in planta*. *F. verticillioides* genes that serve to augment maize host defenses are likely to exhibit patterns of expression that are correlated with exposure to fungal competitors. We have designed a NimbleGen Systems microarray based on over 25,000 *A. flavus* and *F. verticillioides* genes derived from genomic and EST data. Each probe set consists of up to twelve 60-mer oligonucleotides specific to each gene sequence. This report describes gene expression changes in both *F. verticillioides* and *A. flavus* due to co-culture on a maize based medium.

41. Comparative analysis of secondary metabolism gene clusters from two strains of *Aspergillus fumigatus* and closely related species *Neosartorya fischeri* and *Aspergillus clavatus*. Natalie Fedorova[1], Vinita Joardar[1], Jonathan Crabtree[1], Rama Maiti[1], Paolo Amedeo[1], David Denning[2], Jennifer Wortman[1], Geoffrey Turner[3], and William Nierman[1]. [1]The Institute for Genomic Research, Rockville, MD, USA; [2]School of Medicine and Faculty of Life Sciences, The University of Manchester, Manchester, UK; [3]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK. Email: natalief@tigr.org

Genes responsible for biosynthesis of secondary metabolites are often referred to as the most variable segment of fungal genomes. Comparative analysis of secondary metabolism gene clusters from *A. fumigatus* and the two closely related species confirmed that these organisms are indeed relentlessly updating their repertoire of mycotoxins. Out of 23 clusters identified in *A. fumigatus*, only seven have putative orthologs in the other two aspergilli. Most of these 'core' clusters are highly conserved and appear to be under strong negative selection. The rest of the clusters, however, are species- or even strain-specific, implying that the aspergilli must utilize specific mechanisms to ensure a quick divergence and turnover of clusters in the course of evolution. These mechanisms may involve de-novo assembly, segmental duplication, translocation, accelerated differentiation, and differential loss of clusters in various lineages.

42. Improvements in *Aspergillus fumigatus* annotation. Paolo Amedeo, Natalie Fedorova, Rama Maiti, Vinita Joardar, Crabtree Jonathan, Samuel Angiuoli, William Nierman, Owen White, Jennifer Russo Wortman The Institute for Genomic Research, 9712 Medical Center Dr., Rockville, MD 20850

Aspergillus fumigatus has been one among the first fungal genomes to be fully sequenced and annotated. The almost complete absence of genes belonging to closely-related organisms, in public databases, had influenced negatively the quality of annotation. Recently, several other *Aspergillus* species have been fully sequenced and annotated. Moreover, during these past years, annotation tools have been considerably improved. Leveraging on comparative genomic analysis we have revised the annotation of this genome. Here we describe the process taken and demonstrate the improvements.

43. Metabolic network-driven analysis of genome-wide transcription data from *Aspergillus nidulans*. Helga Moreira David[1], Gerald Hofmann[2], Ana Paula Oliveira[2], Hanne Jarmer[3], and Jens Nielsen[2]. [1]Fluxome Sciences A/S, Diplomvej 378, 2800 Lyngby, Denmark [2] Center for Microbial Biotechnology, Technical University of Denmark, 2800 Lyngby, Denmark [3] Center for Biological Sequence Analysis, Technical University of Denmark, 2800 Lyngby, Denmark

Aspergillus nidulans is a model organism for aspergilli, an important group of filamentous fungi that encompasses human and plant pathogens, as well as industrial cell factories. Aspergilli have a highly diversified metabolism and, because of their medical, agricultural and biotechnological importance, it is valuable to understand how their metabolism is regulated. We therefore performed genome-wide transcription analysis of *A. nidulans* grown on glucose, glycerol, and ethanol with the objective of identifying global regulatory structures. We furthermore reconstructed the complete metabolic network of this organism, which resulted in linking 666 genes to metabolic functions, as well as assigning metabolic roles to 472 genes that were previously uncharacterized. Through combination of the reconstructed metabolic network and the transcription data, we identified subnetwork structures that pointed to coordinated regulation of genes involved in many different parts of the metabolism. Thus, for a shift from glucose to ethanol, we identified a coordinated regulation of the complete pathway for oxidation of ethanol, as well as up-regulation of gluconeogenesis and down-regulation of glycolysis and the Pentose Phosphate (PP) pathway. Furthermore, upon a change in the carbon source from glucose to ethanol, the cells shift from using the PP pathway as the major source of NADPH for biosynthesis to use of the malic enzyme. Our analysis indicated that some of the genes are regulated by common transcription factors, making it possible to establish new putative links between known transcription factors and genes, through clustering.

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FUNGAL-HOST INTERACTIONS

44. Translating and Coping with Stress: eIF2? kinases and the Cross-Pathway Control System of *Aspergillus fumigatus*. Christoph Sasse[1], Elaine M. Bignell[2], Stanley Kim[3], Gerhard H. Braus[1], Sven Krappmann[1]. [1]Georg-August University Goettingen, Grisebachstr. 8, D-37077 Goettingen, Germany; [2]Imperial College London, UK; [3]The Institute for Genomic Research, USA

Aspergilli represent unique pathogens with *Aspergillus fumigatus* being the predominant perpetrator. We are interested in nutritional requirements sustaining propagation and supporting virulence. Fungal amino acid (aa) biosynthesis is regulated on various levels; besides pathway-specific systems, one global regulatory network has evolved that acts on aa metabolism as a whole. In its very components, this *C*ross-*P*athway *C*ontrol is made up by an eIF2 kinase sensing aa deprivation and translating it into increased levels of the transcriptional activator protein CpcA, which in turn elevates transcription for the majority of amino acid biosynthetic genes. As deduced from profiling studies, the scope of the CpcA-directed transcriptome exceeds amino acid biosynthesis. To elucidate the role of the CPC signal transduction cascade, the gene of the sensor kinase CpcC was cloned. In contrast to *cpcAdelta* mutants, strains deleted for *cpcC* are not impaired in virulence, indicating that the basal expression level of CpcA is necessary and sufficient to support pathogenesis. Western blot analyses indicate that the *cpcC*-encoded kinase is not required exclusively to phosphorylate the eIF2 subunit. Upon inspection of the *A. fumigatus* genome, the presence of a related gene (*ifkB*, for initiation factor kinase) could be revealed. The *ifkB* gene was deleted in and corresponding mutant strains are currently evaluated.

45. Defense of *Aspergillus fumigatus* against reactive oxigen species mediated by Afyap1. Franziska Lessing, Olaf Kniemeyer and Axel A. Brakhage. Leibniz Institute for Natural Products and Infection Biology – Hans-Knoell-Institute Friedrich Schiller University, Jena, Germany Contact: franziska.lessing@hki-jena.de

With the increasing number of immunocompromised individuals *Aspergillus fumigatus* has become one of the most important opportunistic fungal pathogens. During infection *A. fumigatus* is confronted with a number of defence mechanisms in the host, particulary neutrophiles and macrophages, which kill conidia by producing reactive oxygen species. We identified a homologe of the AP1 like transcription factor *Yap1* from yeast in *A. fumigatus* which we designated *Afyap1*. In yeast, Yap1p was found to be a global regulator for oxidative stress response and required for the protection of the cell against H_2O_2 and other reactive oxygen species. Yap1 is transported in and out the nucleus under nonstressed conditions. The nuclear export is inhibited by oxidative stress and Yap1 induces the transcription of target genes. Nuclear loaclisation of an Afyap1-eGFP fusion in *A. fumigatus* was dependent on the presence of H_2O_2 and diamide. To identify new targets of *Afyap1*, we compared the proteome pattern from H_2O_2 induced and uninduced wild-type mycelia and a Afyap1 deletion strain by 2D-gel analysis.

46. Proteome analysis of the response of *Aspergillus fumigatus* to iron limitation. André D. Schmidt[1], Olaf Kniemeyer[1], Hubertus Haas[2] and Axel A. Brakhage[1]. [1]Leibniz Institute for Natural Product Research and Infection Biology (HKI) / Friedrich-Schiller-University Jena, Germany [2]Division of Molecular Biology/Biocenter, Innsbruck Medical University, Austria

The acquisition of iron is known to be an essential step in any microbial infection process due to iron-limiting conditions in the human host. This iron limitation is caused by high-affinity iron-binding proteins like transferrin or lactoferrin in the host. Since iron plays an essential role in key metabolic processes like DNA synthesis, oxidative phosphorylation or electron transport *A. fumigatus* has to overcome the iron deficiency by the synthesis of siderophores, which chelate iron. It was shown that an *A. fumigatus* strain unable to synthesize siderophores was attenuated in virulence in a murine infection model. To understand the cellular processes, induced by iron starvation, we analysed the proteome of *A. fumigatus* strain ATCC 46645 grown under iron-deficiency conditions. Under iron depletion, proteins involved in siderophore biosynthesis are upregulated, e.g. L-ornithine N^5-oxygenase (SidA), and iron cluster-containing proteins as aconitase or 3-isopropylmalate dehydratase are down-regulated. In addition, proteins involved in the heme biosynthesis are less abundant under iron-deficiency. Further proteins analysed under different non-linear pH-scales will be presented and their putative role will be discussed.

47. Putative G protein-coupled receptors GPRC and GPRD are involved in development and morphogenesis in *Aspergillus fumigatus.* Alexander Gehrke[1], Thorsten Heinekamp[1] and Axel A. Brakhage[1,2]. [1]Leibniz Institute for Natural Product Research and Infection Biology - Department of Molecular and Applied Microbiology - Hans-Knoell-Institute, [2]Friedrich-Schiller-University, Jena; eMail:alexander.gehrke@hki-jena.de

The opportunistic human-pathogen *Aspergillus fumigatus* was subject to recent studies on cAMP signal transduction with regard to morphogenesis and virulence. To date, one of the most important questions is still unanswered: what are the external signals and the corresponding proteins sensing those ligands or stimuli which enable the fungus to grow in a wide variety of different ecological niches? In a first approach, two genes encoding putative G protein-coupled receptors *gprC* and *gprD*, designated as carbon source-sensing receptors, were deleted in *A. fumigatus*. The physiological characterisation of the mutants revealed altered growth on solid media. However, various growth conditions, which included the use of different carbon- and nitrogen-sources, did not restore the defect of the mutants. Virulence of the mutant strains, as tested in a low-dose murine infection model, was attenuated. The function of the putative GPCRs was further investigated by analysing fluorescent protein-fusions *in vivo* by confocal microscopy. Recent data on the function of the receptors will be presented.

48. Transcriptional profile of *Aspergillus fumigatus* conidia in response to human neutrophils. Sugui, J.[1], Zarember, K.[1], Kim, S.[2], Chang, Y.[1], Nierman, W.[2], Gallin, J.[1] and Kwon-Chung, K.[1]. [1]NIH/NIAID, Bethesda, MD 20892 and [2]The Institute for Genomic Research, Rockville, MD 20850.

Neutrophils are cells of the innate immune system recruited to the sites of infection to phagocytose and kill microbial pathogens. Phagocytosis triggers the production of reactive oxygen species (ROS) as well as the fusion of cytoplasmic granules with the phagolysosome. We have previously shown that neutrophils from CGD patients, which generate little to no ROS, are capable of inhibiting *Aspergillus fumigatus* (AF) conidial growth as efficiently as neutrophils from healthy individuals. This suggests that the inhibition depends on non-oxidative mechanisms. We challenged conidia with normal and CGD neutrophils and analyzed the transcriptional profile. Analysis of the microarray data identified a group of 245 genes that were up-regulated in response to neutrophils. The majority of these genes were up-regulated in conidia but not in hyphae. A general classification of these genes showed that 26% are involved in transport, 17% in transcriptional regulation, 13% are related to peroxisomes, 12% in C1-C3 metabolism and 9% in tricarboxylic/glyoxylate cycles. The increase in sugar transport and C1-C3 carbon metabolism suggests an increased requirement of carbon sources when conidia are challenged with neutrophils and fatty acids might be used as carbon source via the tricarboxylic and glyoxylate cycles. Real time PCR data confirmed the up-regulation of 3 genes involved in C1-C3 metabolism; isocitrate lyase (a key enzyme of the glyoxylate cycle), a peroxisomal biogenesis factor and a NAD-dependent formate dehydrogenase. Studies are underway to investigate the biology of AF conidia inside human neutrophils.

GENOME STRUCTURE AND MAINTENANCE

49. Transcriptome analysis of *Aspergillus nidulans* exposed to camptothecin-induced DNA damage. Iran Malavazi[1], Marcela Savoldi[1], Sônia Marli Zingaretti Di Mauro[2], Carlos Frederico Martins Menck[3], Steven D. Harris[4], Maria Helena de Souza Goldman[5], and Gustavo Henrique Goldman[1]. [1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil [2] Universidade de Ribeirão Preto, São Paulo, Brazil, [3] Instituto de Ciências Biomédicas, Universidade de São Paulo, Brazil, [4] Plant Science Initiative, University of Nebraska, Lincoln, Nebraska, [5]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto Universidade de São Paulo, Brazil.

We have used an *Aspergillus nidulans* macroarray carrying sequences of 2,787 genes from this fungus to monitor gene expression of both wild-type and $uvsB^{ATR}$ deletion strains in a time course exposure to camptothecin (CPT). The results revealed a total of 1,512 and 1,700 genes in the wild-type and $uvsB^{ATR}$ deletion strain respectively that displayed a statistically significant difference in at least one experimental time point. We characterized six genes that have increased mRNA expression in the presence of CPT in the wild-type strain relative to the $uvsB^{ATR}$ mutant strain: *fhdA* (forkhead associated domain protein), *tprA* (hypothetical protein that contains a tetratrico peptide repeat), *mshA* (MutS homologue involved in mismatch repair), *phbA* (prohibitin homologue), $uvsC^{RAD51}$ (RAD51 homologue), and *cshA* (homologue of the excision repair protein ERCC-6 [Cockayne's syndrome protein]). The induced transcript levels of these genes in the presence of CPT require $uvsB^{ATR}$. These genes were deleted, and surprisingly, only the delta *uvsC* mutant strain was sensitive to CPT; however, the others displayed sensitivity to a range of DNA-damaging and oxidative stress agents. These results indicate that the selected genes when inactivated display very complex and heterogeneous sensitivity behavior during growth in the presence of agents that directly or indirectly cause DNA damage. Moreover, with the exception of UvsC, deletion of each of these genes partially suppressed the sensitivity of the delta *uvsB* strain to menadione and paraquat. Our results provide the first insight into the overall complexity of the response to DNA damage in filamentous fungi and suggest that multiple pathways may act in parallel to mediate DNA repair.
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INDUSTRIAL BIOLOGY

50. New screening approaches for fungal strain development. Peter J. Punt[1], Xavier O. Weenink[2], Marc van der Maarel[1], Jan Jore[1], Arthur Ram[2], Cees van den Hondel[2]. [1]TNO Quality of Life, Zeist, the Netherlands,[2]Leiden University, Leiden, the Netherlands

Since the development of recombinant DNA technologies for yeast and filamentous fungi, a considerable part of the strain development programs was diverted to the use of molecular genetic tools. Whereas these approaches have exerted considerable success, recent developments in our laboratory have shown that new developments in classical biological screening approaches, or a combination of both, can still be very useful. A first purely classical approach is based on the discovery of a so-called suicide (SUI) substrate, which we have successfully used for the selection of protease deficient fungal host strains. These protease deficient strains show an increased resistance to the SUI substrate allowing their selection. The advantage of this non-GMO approach is that it can be applied to new and already established production strains. A combination of a molecular and classical approach is based on the use of the so-called glucoamylase carrier approach. Combining this approach with fungal strains unable to use starch as a carbon source allowed us to select for hyper secretive fungal strains generated by classical mutagenesis (Weenink et al., 2006). Moreover, the same approach also allows for selection of the highest producers in a collection of primary transformant strains expressing a glucoamylase-fusion gene.

51. Engineering of a novel protein secretion pathway in *Aspergillus niger*. Robbert Damveld[1], Miranda Hartog[1], Peter ten Haaft[1], Inge Minneboo[1], Cees Sagt[1], Han de Winde[1], Thibaut Wenzel[1]. [1]DSM Food Specialties, P.O. Box 1, 2600 MA Delft, The Netherlands.

Production of enzymes on an industrial scale is often limited to extracellularly enzymes. When enzymes are produced intracellular, the downstream processing is costly and process robustness is not guaranteed. We have developed a technology which enables the secretion of enzymes which are normally localized in the cell. The folding conditions of intracellular proteins differ from those of secreted proteins. In the cytosol proteins fold under relative reducing conditions compared to the oxidizing environment in the ER. Moreover, many secreted proteins undergo extensive N- glycosylation and disulphide bridge formation. The set of folding enzymes and chaperones in the secretory pathway and in the cytosol are different as well. Therefore the simple solution of forcing intracellular proteins through the secretory pathway does in many cases fail to result in biologically active secreted enzymes. We have developed a method to secrete intracellular enzymes in an active form. This technology allows the intracellular enzymes to be folded in their native environment in the cytosol using cytosolic folding enzymes and chaperones. After folding the intracellular proteins are translocated to a modified cell compartment. After translocation in this modified cell compartment the content is released into the medium by a specific process. Using controlled fermentations we have established that the physiology of cells containing this novel secretory system is not dramatically different form non-modified cells. We will show the recently obtained proof of principle of this concept with the Green Fluorescent Protein (GFP). In addition we have demonstrated the concept for intracellular enzymes, which are secreted in an active form using this novel approach. Moreover we also have preliminary evidence that this technology can be used to secrete metabolites, which are normally localized intracellular. This is the first report that describes the introduction of a completely novel secretory pathway in eukaryotic cells, which allows the production of intracellular enzymes in a secreted active form enabling industrial application on an economically feasible scale.

52. Applied genome-scale modelling of *Aspergillus niger*. Mikael Rørdam Andersen, Michael Lynge Nielsen, Jens Nielsen. Center for Microbial Biotechnology, Build. 223, Technical University of Denmark, DK-2800 Lyngby, Denmark, jn@biocentrum.dtu.dk

The filamentous fungus *Aspergillus niger* has through the years fascinated academic and industrial researchers alike due to its innate ability to produce a large number of enzymes and high concentrations of acids. However, the regulatory mechanisms governing these processes are not thoroughly understood. We are combining genome-scale stoichiometric modeling, transcriptomics and novel tools for graphical analysis to elucidate these mechanisms.
A genome-scale model was constructed based on literature of *A. niger* and related aspergilli. Completion of pathways was performed using pathway databases. Genomic information was added based on the sequencing and annotation provided by DSM Food Specialities (Pel *et al*, manuscript accepted). The model comprises 1230 biochemically unique reactions (isoenzymes not included). 850 are supported by literature and 873 are backed by the genomic evidence of 1024 open reading frames. The remaining reactions are transport reactions and other reactions added for connectivity. Using a digital pathway map of the model, the computed metabolic fluxes were analysed for a large number of substrates. The results are in accordance with the available literature.
Using a fast PCR-based cloning-free approach (Nielsen *et al,* 2006) adapted for *A. niger*, deletion mutants of carbon metabolism regulators of *A. niger* have been constructed. These are to be analysed using steady-state cultures and DNA arrays for *A. niger*.

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53. A transiently disrupted non-homologous end joining pathway in *Aspergillus nidulans* allows simple and efficient gene targeting. Jakob Blæsbjerg Nielsen, Michael Lynge Nielsen, and Uffe Hasbro Mortensen. Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark, Denmark jbn@biocentrum.dtu.dk

Gene targeting was developed more than a decade ago for high-throughput gene deletions in the unicellular *Saccharomyces cerevisiae* owing to the fact that DNA double strand breaks are effectively repaired by the homologous recombination (HR) pathway. In multicellular eukaryotes, from filamentous fungi to man, DNA double strand breaks are preferentially repaired by the non-homologous end joining (NHEJ) repair pathway. When performing gene targeting in such multicellular organisms, the influence of NHEJ compromises the efficiency by causing random integration of the gene targeting DNA. Elimination of NHEJ by deleting one of the main genes in the pathway, e.g. *ku70*, *ku80* or *lig4* has been done in several fungal species including *Aspergillus fumigatus*, *A. nidulans*, *Sordaria macrospora* and *Neurospora crassa*. In such strains, the gene targeting efficiency is often as high as 100%. The phenotype of NHEJ strains includes altered colony morphology and an increase in sensitivity to various genotoxins. This raises the concern that phenotype of NHEJ deficient strains may produce synthetic effects with other mutations introduced in this background. This may hamper direct interpretation of the phenotype of this new mutation. In line with this, authors often recommend as a safety measure to reconstitute the NHEJ pathway, e.g. by retransforming with the functional gene or sexual crossing with wild-type strains, before analyzing the effects of novel mutations. In both cases, strain reconstruction constitutes a bottle neck in large scale gene-targeting experiments. To bypass these problems, we have developed an effective alternative in *A. nidulans*. The system employs a strain with a transiently disrupted *ku70* homolog as the starting point for efficient gene targeting. The *ku70* mutation in this strain can via a simple selection scheme be reverted to wild type after the desired genetic manipulation(s) have been carried out. The system can easily be adapted to other filamentous fungi.

54. Isolation of isoosmotic up-regulated genes in *Aspergillus oryzae* and use of its promoters for protein expression system. Ken Oda[1], Kazutoshi Sakamoto[2], Toshihide Arima[3], Yuka Okita[2], Dararat Kakizono[2], Osamu Yamada[2], Shinichi Ohashi[1], Osamu Akita[5], Kazuhiro Iwashita[2,4]. Kanazawa Institute of Technology[1],3-1 Yatsukaho, Hakusan, Ishikawa, Japan tel D+81(76)274-7500, fax+81(76)274-7511, e-mail Fodaken@neptune.kanazawa-it.ac.jp National Research Institute of Brewing[2], Prefecture Univ. of Hiroshima[3], Hiroshima Univ.[4], Jissen Women's Univ.[5], Japan

The mechanism of response to osmotic pressure exists in eukaryotes. In *A. orzyae*, response to osmotic pressure is significant factor for environment recognition in koji-making (solid-state culture), and in fact it was suggested that osmotic pressure regulation component, such as *AtfA*, or *HogA*, is important. We analyzed the genes which respond to isoosmotic pressure (0.8M NaCl and 1.2M sorbitol) by microarray analysis. In isoosmotic condition 96 genes in 3000 spots array were 2-fold up- regulated from 30 to 90 min after induction. Some genes that are needed to respond to osmotic stress, such as *atfA*, , *gpd*, , and *srk1*, were isolated. 19 genes were over 5-fold up-regulated, and named as Isoosmotic Up-regulated Genes (IUG). 5Õ- upstream region of 10 genes in IUG were cloned and applied to GUS reporter assay to evaluate induction ability in isoosmotic condition. The promoters of two genes (IUG2 and IUG9) were strictly regulated. To construct protein expression system the promoter region of high expression vector pNGA142 was substituted with IUG2 and IUG9 promoters. EGFP as a model of heterologous protein was expressed after 2hr only in induction conditions, suggesting that response speed of this system is fast and control of expression is strict. We constructed new protein expression system which protein expression was induced by osmotic pressure and their level of expression are controlled by NaCl concentration.

55. Highly efficient gene targeting in *Aspergillus oryzae* deficient in DNA ligase IV ortholog (LigD). Youhei Kudo[1], Osamu Mizutani[1], Akemi Saito[1], Tomomi Matsuura[1], Hirokazu Inoue[2], Keietsu Abe[1], Katsuya Gomi[1]. [1]Graduate School of Agricultural Science, Tohoku University, Sendai, [2]Faculty of Science, Saitama University, Saitama, Japan. gomi@biochem.tohoku.ac.jp

Disruption of the gene that encodes Ku70 or Ku80 involved in the non-homologous end joining (NHEJ) has significantly increased the frequency of gene targeting in filamentous fungi, and thus the mutants deficient in Ku70/Ku80 are suitable hosts for comprehensive gene knockout. However, even though using *ku70/ku80* mutant, we sometimes failed to obtain an expected gene disruptant in *Aspergillus oryzae*, probably dependent on the gene of interest. Thus, we constructed the disruptant deficient in DNA ligase IV ortholog (LigD) involved in the final step of NHEJ in *A. oryzae* and examined the effect of *ligD* disruption on gene targeting frequency. The *ligD* disruptant showed no apparent growth defect and a similar sensitivity to DNA-damaging agents. Gene replacement of the *prtR* gene encoding a transcription factor for extracellular proteolytic genes using *A. nidulans sC* gene as a selectable marker resulted in 100% of gene targeting frequency in the *ligD* disruptant. In addition, gene replacement of five MAP kinase genes found in *A. oryzae* genome database also showed the targeting rates as high as 100%. Consequently, the *ligD* deletion mutants are quite excellent tools for gene targeting in *A. oryzae*.

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OTHER

56. Analysis of four putative beta–oxidation genes in *Aspergillus nidulans*. Kathrin Reiser, Meryl A. Davis and Michael J. Hynes. Department of Genetics, University of Melbourne, Parkville 3010, Australia

Filamentous fungi are able to use fatty acids as sole carbon sources via beta-oxidation. The enzymes required are present in both peroxisomes and mitochondria (Maggio-Hall and Keller 2004 Mol. Microbiol. 54:1173–1185).
Two putative fatty acyl-CoA dehydrogenases, AcdA and AcdB, and two putative fatty acyl-CoA oxidases, AoxA and AoxB, were identified in the genome of *A. nidulans*. Because of their homology to Fox1p of *S. cerevisiae* and a peroxisomal dehydrogenase of *N. crassa*, they are thought to be involved in the first step of beta-oxidation. Homologues for each were found in other fungal species. Three of the proteins have a clear PTS1 (peroxisomal targeting sequence), while AcdA has a PTS1-like sequence. Hence, all four proteins are predicted to be peroxisomal. GFP fusion proteins have been constructed to investigate this hypothesis.
In the 5' promoter region (1kb) of each gene, a 6bp sequence (CCGAGG/ CCTCGG) was found at least once. This sequence is predicted to be the core of a fatty acid depending regulation site (Hynes et al. 2006 Eukaryot. Cell. 5:794- 805) suggesting positive regulation by fatty acids. Promoter-lacZ fusions have shown that at least two genes are induced by short- and long-chain fatty acids and induction is altered in the absence of the predicted regulatory proteins.
Deletions of all four genes do not give a severe fatty acid growth phenotype. Only the *aox*A-deletion phenotype is clearly visible. This implies a great redundancy amongst peroxisomal proteins involved in the first step of beta- oxidation.

57. Mitotic recombination accelerates adaptation in *Aspergillus nidulans*. Sijmen Schoustra, Fons Debets, Marijke Slakhorst & Rolf Hoekstra, Laboratory of Genetics, Wageningen University, e- mail: rolf.hoekstra@wur.nl

We have performed an experimental study to explore the specific advantages of haploidy or diploidy in the fungus *Aspergillus nidulans*. Comparing the rate of adaptation to a novel environment between haploid and isogenic diploid strains over 3000 mitotic generations, we demonstrate that diploid strains which during the experiment have reverted to haploidy following parasexual recombination reach the highest fitness. This is due to the accumulation of recessive deleterious mutations in diploid nuclei, some of which show their combined beneficial effect in haploid recombinants. Our findings show the adaptive significance of mitotic recombination combined with a flexibility in the timing of ploidy level transition if sign epistasis is an important determinant of fitness. We believe that our results justify a rehabilitation of Pontecorvo's view that the parasexual cycle has an important evolutionary role in fungi, because we show that in initially homozygous diploids sufficient genetic variation is generated by mutation to make parasexual recombination effective.

58. Identification of AatB, a new component of the penicillin biosynthesis pathway of *Aspergillus nidulans*. Petra Sproete [1], Michael J. Hynes [2], and Axel A. Brakhage [1]. [1] Leibniz Institute for Natural Product Research and Infection Biology (HKI) / Friedrich-Schiller-University Jena, Germany [2] Department of Genetics, University of Melbourne, Australia

The acyl coenzyme A:isopenicillin N acyltransferase (IAT) of *A. nidulans*, which is encoded by the *aatA* gene, catalyzes the final step of the penicillin biosynthesis, i.e., the exchange of the hydrophilic L-alpha-aminoadipic acid side chain of isopenicillin N for a hydrophobic acyl group. By analyzing a GFP-IAT protein fusion it could be shown that in *A. nidulans* – as in *Penicillium chrysogenum* – the IAT and therefore the enzymatic reaction is located in the peroxisomes. Further studies indicated a PTS1 dependent transport of the enzyme since the deletion of the rather untypical putative peroxisomal targeting sequence 1 (PTS1) Ala-Asn-Ile at the C terminus of the protein led to cytoplasmic localization of the IAT. Nevertheless, unlike the IAT of *P. chrysogenum*, such a mislocated enzyme seems to be functional because both, an *A. nidulans* strain lacking the PTS1 transporter and a strain possessing a mislocated IAT still produced about 50% and 80% of penicillin, respectively, compared to wild-type levels. Because an *aatA* disruption strain still was able to produce small amounts of an inhibitory substance, the *A. nidulans* database was searched for a putative redundant protein. A gene displaying a very similar exon distribution and a 58% similarity with the *aatA* gene but lacking the PTS1 encoding sequence was named *aatB*. First analyses of an *aatB* disruption strain indicated a participitation of AatB in penicillin biosynthesis of *A. nidulans*.

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59. CoA-Transferase from *Aspergillus nidulans* is required to forward the CoA-moiety from propionyl-CoA to acetate. Christian Fleck, Matthias Brock. Leibniz Institute for Natural Product Research and Infection Biology e.V. – Hans-Knöll-Institute (HKI) Contact: christian.fleck@hki-jena.de

Metabolism of amino acids and propionate leads to the toxic intermediate propionyl-CoA. Efficient removal via the methylcitrate cycle is guaranteed by a functional methylcitrate synthase. Deletion of the coding region of this enzyme leads to the inability to grow on propionate or ethanol/propionate but not acetate/propionate. This led to the assumption that the level of propionyl-CoA may become reduced by direct transfer of the CoA-moiety to acetate. Therefore, we purified a CoA-transferase from *Aspergillus nidulans* and characterised the enzyme biochemically. The CoA-transferase was specific for the CoA-esters succinyl-CoA, propionyl-CoA, and acetyl-CoA as CoA-donors and the corresponding acids as acceptors. No other donors or acceptors suited as substrates. To elucidate the role of the enzyme under *in vivo* conditions the corresponding gene was deleted. The deletion mutant only showed mild phenotypes when tested for growth and development on propionate containing media. Therefore, a double mutant with a methylcitrate synthase deletion strain was created by sexual crossing. In contrast to the methylcitrate synthase deletion strain, the double mutant was neither able to grow on ethanol/propionate nor on acetate/propionate media. Thus, CoA-transferase is essential for removal of toxic propionyl-CoA in the presence of acetate as a CoA-acceptor.

60. Gene Silencing by RNA Interference in the Koji Mold *Aspergillus oryzae*. Osamu Yamada, Ryoko Ikeda, Yuka Ohkita, Risa Hayashi, Kazutoshi Sakamoto, and Osamu Akita. National Research Institute of Brewing, 3-7-1 Kagamiyama, Higashi-Hiroshima 739-0046, Japan

Aspergillus oryzae is an important filamentous fungus in the Japanese fermentation industry, used in the manufacture of such products as sake, soy sauce, and miso, as well as in commercial enzyme production. We found the orthologous genes required for RNA interference (RNAi) in the *A. oryzae* genome database, and constructed a set of tools for gene silencing using RNAi. This system utilizes compatible restriction enzyme sites so that only a single target gene fragment is required to create the hairpin RNA cassette. For ease of handling, we also separated the construction of the hairpin RNA cassette for the target gene from its subsequent introduction into the expression vector. Using the *brlA* gene as a target for RNAi, we detected decreased mRNA levels and a delayed conidiation phenotype in the transformants. Furthermore, even though *A. oryzae* possesses three copies of the Taka- amylase gene, a single copy of a Taka-amylase RNAi construct was sufficient to downregulate the mRNA levels and decrease the enzymatic activity to 10% of control levels. Gene silencing by RNAi should provide a powerful genetic tool for post-genomic studies of the industrially important fungus *A. oryzae*.

61. Easy ways to identify *veA1* mutation in *Aspergillus nidulans*. Kap-Hoon Han[1], Hyoun-Young Kim[1], Jong Hwa Kim[1], Hee-Seo Kim[2], Keon-Sang Chae[2] and Dong-Min Han[3]. [1]Dept. of Pharmaceutical Engineering, Woosuk Univ, Wanju, 565-701, [2]Div. of Biological Science, Chonbuk Nat'l Univ, Jeonju, 561-756, [3]Dept. of Life Science, Wonkwang Univ, Iksan, 570-749, Korea.

The *veA* gene in *Aspergillus nidulans* plays an important role in light response and the balance of sexual and asexual sporulation. So, the presence of the *veA1* mutation gave us difficulty to study sexual development as well as photobiological research. Because of the *veA1* mutation was caused by a single nucleotide substitution, nucleotide sequencing of the region was the only way for the verification. Here, we present a few simple ways to identify the *veA* allele easier than the genome sequencing. First, incubation of the *A. nidulans* strains on the medium containing oxalic acid provides clear colony distinguishment between the *veA*+ and *veA1* strain. Secondly, PCR mediated verification allowed us to distinguish more clearly. The PCR products could only be obtained in the wild type *veA* allele using double mismatched primers. Finally, restriction enzyme digestion followed by the PCR amplification of the *veA* allele resulted in the clearest result. Since the *veA1* mutation eliminated a restriction enzyme site, only the PCR product containing *veA*+ allele is subjected to be digested by the enzyme. These methods provided fast and cost-saving ways for identifying the *veA* allele.

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62. Analysis of *pceA*, a regulatory gene for early stage of conidiation in *Aspergillus oryzae*. Kenichiro Matsushima[1], Osamu Hatamoto[2], Genryou Umitsuki[1] and Yasuji Koyama[1]. [1]Noda Institute for Scientific Research, [2]Product Development Division, Kikkoman Corporation. Japan.

The *pceA* gene of *A. oryzae*, encoding a putative transcription factor with a GAL4-like Zn(II)2Cys6 motif, is an ortholog of the *A. nidulans nosA* gene, which is involved in regulation of sexual development. KN16-10, a *pceA* overexpressing strain of *A. oryzae*, exhibited an earlier and increased conidiation compared to the wild-type strain. In addition, KN16-10 produced conidiospores in liquid medium, whereas the wild-type strain did not produce them under the same conditions. The expression of conidiation-related gene homologs, such as *brlA* and *flbA*, and that of the *pceB*, a paralog of *pceA*, were investigated in KN16-10 and the wild-type strain. The Real-Time PCR analysis showed that expression of *brlA*, a regulatory gene for the early stage of conidiation in *A. nidulans*, increased in KN16-10 both in liquid and solid medium. At the same time, the expression of *pceB* increased in parallel with the expression of *pceA*. These findings suggest that *pceA* plays an important regulatory role in the early stage of conidiation in *A. oryzae* by upregulating the expression of *brlA*. Finally, considering that the expression of *pceB* seems to be also upregulated by *pceA*, *pceB* might be involved in conidiation as well, although its exact function remains unclear.

63. Functional analysis of the putative transcription factor CrzA in *Aspergillus nidulans*. A. Spielvogel[1,2], U. Stahl[1], E.A. Espeso[2],V. Meyer[1]. [1]Technische Universität Berlin, Institut für Biotechnologie, Fachgebiet Mikrobiologie und Genetik, 13355 Berlin, Germany [2]Departamento de Microbiología Molecular, Centro de Investigaciones Biológicas CSIC, Madrid 28040, Spain A.Spielvogel@lb.tu-berlin.de

Calcium signaling mechanisms are employed by eukaryotic cells to regulate gene expression in response to various environmental stimuli. In *Saccharomyces cerevisiae*, the Ca2+/calmodulin regulated protein phosphatase calcineurin promotes yeast survival during stress conditions such as alkaline pH, salinity, ER stress and high temperature. The transcription factor Crz1p is a major mediator of calcineurin-dependent genes and regulates different proteins that participate in ion homeostasis, cell wall integrity, membrane trafficking and signal transduction. Crz1p is a C2H2 zinc-finger protein and binds to specific elements (CDRE) within the promoter regions of target genes. Using a reverse genetics approach, we searched the *A. nidulans* genome for the presence of a Crz1p homologue and identified an ORF that displays 66% identity over the zinc-finger region of Crz1p. Based on this sequence similarity, we designated the corresponding gene *crzA*. The zinc-finger domain of CrzA contains three zinc-finger motifs, whereby the first two meet the C2H2 consensus. The entire zinc-finger domain is able to form specific DNA-protein complexes with promoter fragments harbouring CDRE's, indicating that CDRE's represent binding sites that are conserved among yeast and filamentous fungi. Using site-directed mutagenesis, we could show that the non-consensus zinc-finger three is essential for efficient DNA binding. Deletion of *crzA* had no influence on the viability of *A. nidulans*, but rendered the strain sensitive towards calcium, salt stress (Na+, K+) and alkaline pH, indicating that CrzA plays a crucial role in stress adaptation. Northern analyses are currently underway to identify genes regulated by CrzA in response to salt and alkaline stress.

64. Deleting *Aspergillus nidulans* checkpoint regulators in an undergraduate molecular genetics course. Steve James. Biology Department, Gettysburg College, Gettysburg PA sjames@gettysburg.edu

Recent advances in gene targeting and fusion PCR technology make it feasible for undergraduates to design, perform, and characterize gene deletions in filamentous fungi as part of a semester-long laboratory course. For the past two years, undergraduates in the Molecular Genetics course at Gettysburg College have deleted *Aspergillus nidulans* genes involved in cell cycle checkpoint control. Working in pairs, students applied bioinformatic tools to design a set of six PCR primers for creating a gene replacement construct. They used these primers in three-way fusion PCR to join the *A. fumigatus pyrG* selectable marker with sequences flanking the target gene. In subsequent steps, students (1) generated *pyr+* transformants in strains bearing a deletion of the KU70 homolog, (2) isolated genomic DNA and performed Southern blots using *pyrG A.f.* as a probe, (3) tested their targeted deletions for increased sensitivity to genotoxic agents, and (4) amplified and cloned the coding region into an *alcA*-based overexpression vector. Students completed this capstone experience by writing an as-for- publication paper in which they used bioinformatic tools and current literature to integrate their findings with the broader fields of cell cycle regulation and DNA damage checkpoint control. As well as providing rich opportunities for original, investigative research, this course serves as a wellspring of new projects for students wishing to continue into independent research. (Supported by Gettysburg College)

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65. Aspergillus at the FGSC: Expanding with the community. Kevin McCluskey, Sheera Walker and Aric Wiest. Fungal Genetics Stock Center, University of Missouri- Kansas City, School of Biological Sciences

The Fungal Genetics Stock Center has endeavored to support research efforts with a number of fungi for almost fifty years. The first *Aspergillus nidulans* strain was deposited in October of 1962. In 2007 the FGSC holdings totaled include nearly 900 *A. nidulans* strains and 129 are *A. niger*. Recent deposits have included many *A. fumigatus* strains as well as several strains from genome sequencing programs. 37 of the genetically marked Aspergillus strains in the FGSC collection have only one marker while 178 strains have two markers (including veA+). 260 strains have three markers while 168 strains have 4 markers. Fifty eight strains have ten or more markers. Two strains share the record of the greatest number of markers with 15 each. (A591 and 593). The average Aspergillus strain has 8 markers and the most common markers are biA1 and yA2. There are 869 different loci represented among strains in the FGSC collection

The experience that the FGSC gains from being part of functional genomics programs makes us uniquely qualified to offer the same services to the Aspergillus community. As new effort is dedicated to unraveling the genomes of Aspergillus species, the FGSC can assure that resources are put to their greatest benefit, drawing new scientists to the field of Aspergillus research. Key technologies include arraying mutants, international distribution and support of information dissemination.

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66. Development and evaluation of an Affymetrix array for *Aspergillus flavus*. Andrea Dolezal[1], David Ryan Georgianna[1], Greg OBrian[1], Charles Woloshuk[2], Nancy Keller[3], Jiujiang Yu[4], Dahlia Nielsen[1], Gary Payne[1]. [1] North Carolina State University, Raleigh, NC. [2] Purdue University, West Lafayette, IN. [3] University of Wisconsin, Madison, WI. [4]USDA/ARS/SRRC, New Orleans, LA.

A multi-species Affymetrix GeneChip array was developed to study development, metabolism, and pathogenicity of *A. flavus*. This chip, based on the whole genome sequence of *A. flavus*, contains 13,000 *A. flavus* genes, 8,000 maize genes, and 25 human and mouse innate immune response genes as well as the fumonisin and trichothecene clusters from *Fusarium*. These arrays were used to monitor gene expression of *A. flavus* during aflatoxin biosynthesis in defined media and during infection of developing maize seeds. A parallel study comparing this array with a 5002 element cDNA array showed the same expression profile for the aflatoxin biosynthetic genes when *A. flavus* was grown in culture on defined media at conducive and non conducive temperatures for aflatoxin production. Gene expression was also monitored in *A. flavus* during the infection of field grown maize seeds. The profile of aflatoxin gene expression by *A. flavus* in infected maize kernels was similar to that observed for *A. flavus* grown in the lab under conductive temperatures for aflatoxin production. In addition, several genes encoding enzymes for the metabolism of complex carbohydrates and for transporters were also elevated during infection of maize seeds. Nonspecific hybridization across species has not been observed in any of our experiments using the Affymetrix GeneChip. These initial observations show that these multi-species arrays will be a powerful tool for studying the complex ecology and metabolism of *A. flavus*. This research was funded by USDA/NRI/CGP 2006-35604-16666.

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24th Fungal Genetics Conference at Asilomar

Community sponsored Workshops

Tuesday, March 20 - 3:00 pm - 5:30 pm
Sclerotinia/Botrytis Annotation meeting
Marty Dickman (mbdickman@neo.tamu.edu)

Wednesday, March 21 - 12:00 - 2:00

Fusarium graminearum Workshop
Frances Trail (trail@msu.edu)

The Phycomyces genome: manual annotation and analysis
Luis Corrochano, corrochano@us.es

Thursday, March 22 - 12:00 - 2:00
Nectria haematococca Sequence
Hans VanEtten (vanetten@Ag.arizona.edu)

Dothideomycete Genome Sequence Workshop
Peter Solomon (P.Solomon@murdoch.edu.au)

Planning Session on functional characterization of orphan genes and hypothetical proteins
Heather Adams (hallenhe@msu.edu)

Friday, March 23 - 12:00 - 2:00
Making Gene Ontology Annotations for Fungal Genomes
Karen Christie (kchris@genome.stanford.edu)

Heterobasidion genome sequence Workshop
Jan Stenlid (Jan.Stenlid@mykopat.slu.se)

Sunday, March 25 - 9:00 - 11:30
Comparative genomics of the fusaria and organization of a Fusarium working group
Frances Trail, Hans VanEtten, Seogchan Kang and Won-Bo Shim

Sunday, March 25 9:00- 5:00
Colletotrichum workshop
LisaVaillancourt (vaillan@uky.edu)
Dov Prusky (dovprusk@volcani.agri.gov.il)

Asilomar Fungal Genetics Meeting 2007
Teaching Fungal Biology and Genetics 3-6 pm, Wed. March 21, 2007
Amy J. Reese and Patricia Pukkila, session co-chairs

Schedule proposal

3:00 – 3:10 Amy J. Reese and Patricia Pukkila, ***Introduction to session***

3:10 – 3:30 Thomas Volk, University of Wisconsin- La Crosse, ***The internet and its ability to lure people into learning something about fungi they didn't know they wanted to know***

3:30 – 3:50 Maria Costanzo, Saccharomyces Genome Database, ***Fungal gene and protein information at the Saccharomyces and Candida Genome Databases***

3:50 – 4:35 Amy J. Reese, Cedar Crest College, ***Fungal genetics and biology round table discussion: best practices and trouble spots***

4:35 – 4:55 Coffee break

4:55 – 5:15 Steven James, Gettysburg College, ***Deleting Aspergillus nidulans checkpoint regulators in an undergraduate molecular genetics course***

5:15 – 5:35 Sarah Lea Mcguire, Millsaps College, ***Teaching with fungi: from college freshmen to seniors***

5:35 – 5:55 Patricia Pukkila, The University of North Carolina at Chapel Hill, ***Bringing student inquiry and research into your courses by collaborating with graduate research consultants or advanced undergraduates***

5:55 – 6:00 Patricia Pukkila, ***Wrap-up to session***

Concurrent Session: Teaching Fungal Biology & Genetics

- If you are interested in receiving notes from this session, please fill out the sign-in form.

Academic roles represented

- Do your roles include:
  - Mostly research
  - Research & teaching
  - Mostly teaching
  - Graduate student / post-doc
  - Patient care
  - Administration
  - Other

Institution types represented

- Would you consider your school to be:
  - Mostly a research university / medical school?
  - A balance between research & teaching?
  - Mostly a teaching college or university?
  - A community or technical college?
  - Non-academic?
  - Other?

Level of fungal topics taught

- Fungal biology/genetics topics covered:
  - At the medical school level
  - At the graduate level
  - Upper undergraduate level
  - General microbiology course
  - Introductory biology course
  - Other

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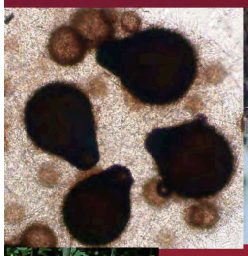
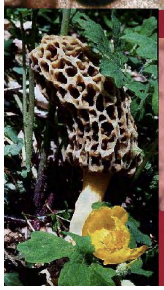
Luring people into learning about Mycology using the internet*

**Even though they didn't know they wanted to learn anything at all*





24th Fungal Genetics Conference
Asilomar, CA
March 21, 2007

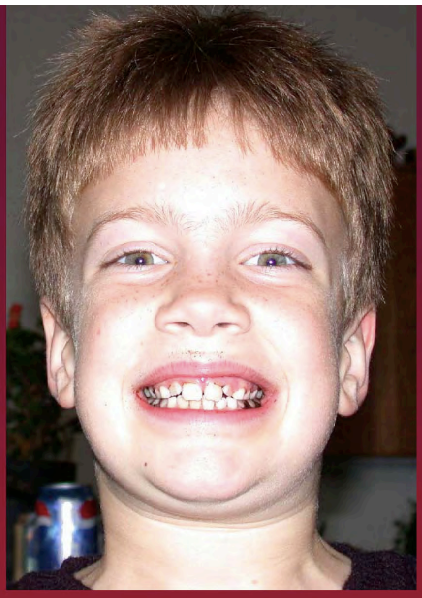
Tom Volk
Department of Biology
University of Wisconsin-La Crosse
TomVolkFungi.net

University of Wisconsin-La Crosse

- On the Mississippi River in western Wisconsin
- About 9000 students
- About 1200 Biology and Microbiology majors

I teach courses in Mycology, Medical Mycology, Plant-Microbe Interactions, Advanced Mycology, Food & Industrial Mycology, Plant Biology, Organismal Biology, Genetics Lab, and Latin & Greek for scientists

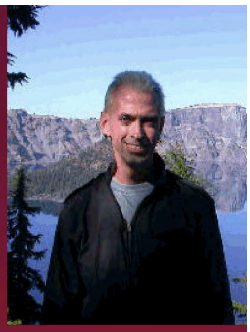
Don't be afraid of Mycology



- Jennifer, Keef and Fungal Genetics

"My mommy works in a Jungle Phonetics lab"

Why have web pages online?



- Most people do not know what Mycology is.
- Email: *I have to do a report on mycology for my science class. Could you please send me a picture of mycology? Thanks.*
- The internet provides an opportunity for educating a large number of people
- Almost 980,000 visitors to my main page at TomVolkFungi.net since I went online in November 1995.
- 10,000-20,000 visitors per month
- Millions more on the rest of my pages

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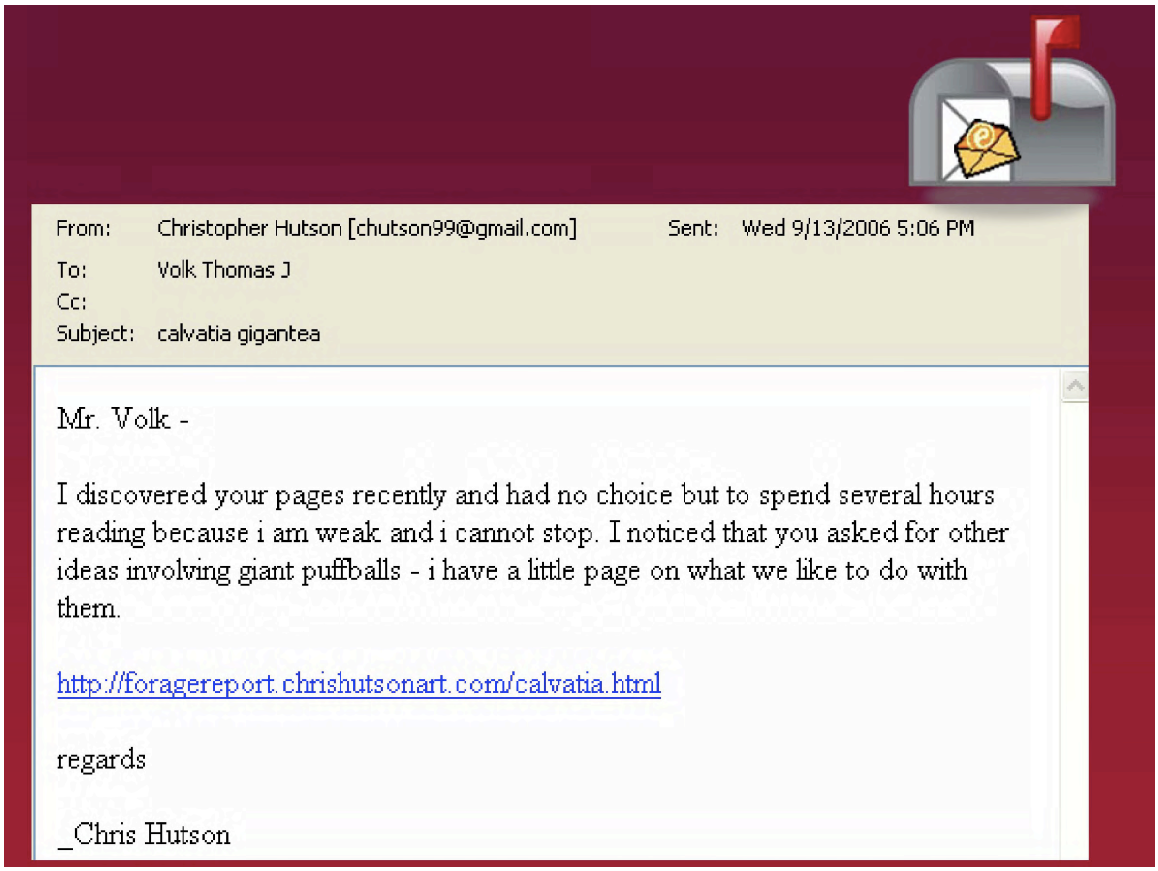

From: Christopher Hutson [chutson99@gmail.com] Sent: Wed 9/13/2006 5:06 PM
To: Volk Thomas J
Cc:
Subject: calvatia gigantea
Mr. Volk -
I discovered your pages recently and had no choice but to spend several hours reading because i am weak and i cannot stop. I noticed that you asked for other ideas involving giant puffballs - i have a little page on what we like to do with them.
http://foragereport.chrishutsonart.com/calvatia.html
regards
_Chris Hutson

Something for everyone?
TomVolkFungi.net
Fungus of the month pages.
Relate fungi to holidays
Relate fungi to everyday life.
e.g. Dog stinkhorn, Stachybotrys
Relate web pages to history
Irish potato famine, Caesar's mushroom
Genetics: Schizophyllum, this month
Gross-out: Dog vomit slime mold, athlete's foot
Fun: Pilobolus

Web pages bring Email

- 75-100 unsolicited emails per week

From: Beth Spencer [ann25spen@yahoo.com] Sent: Tue 9/26/2006 4:11 PM
To: Volk Thomas J
Cc:
Subject:

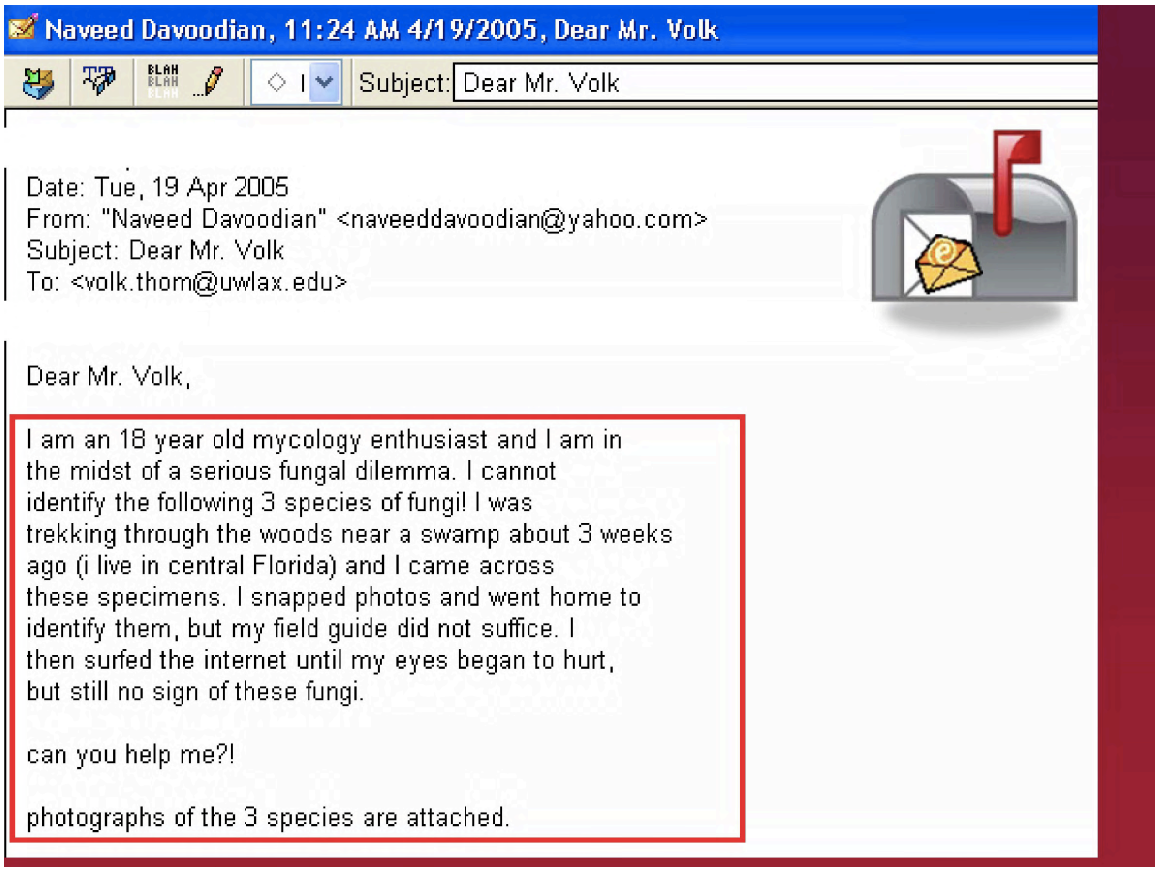
i have a mushroom growing in my yard and wanted to know if i could eat it or what kind it was thanks beth

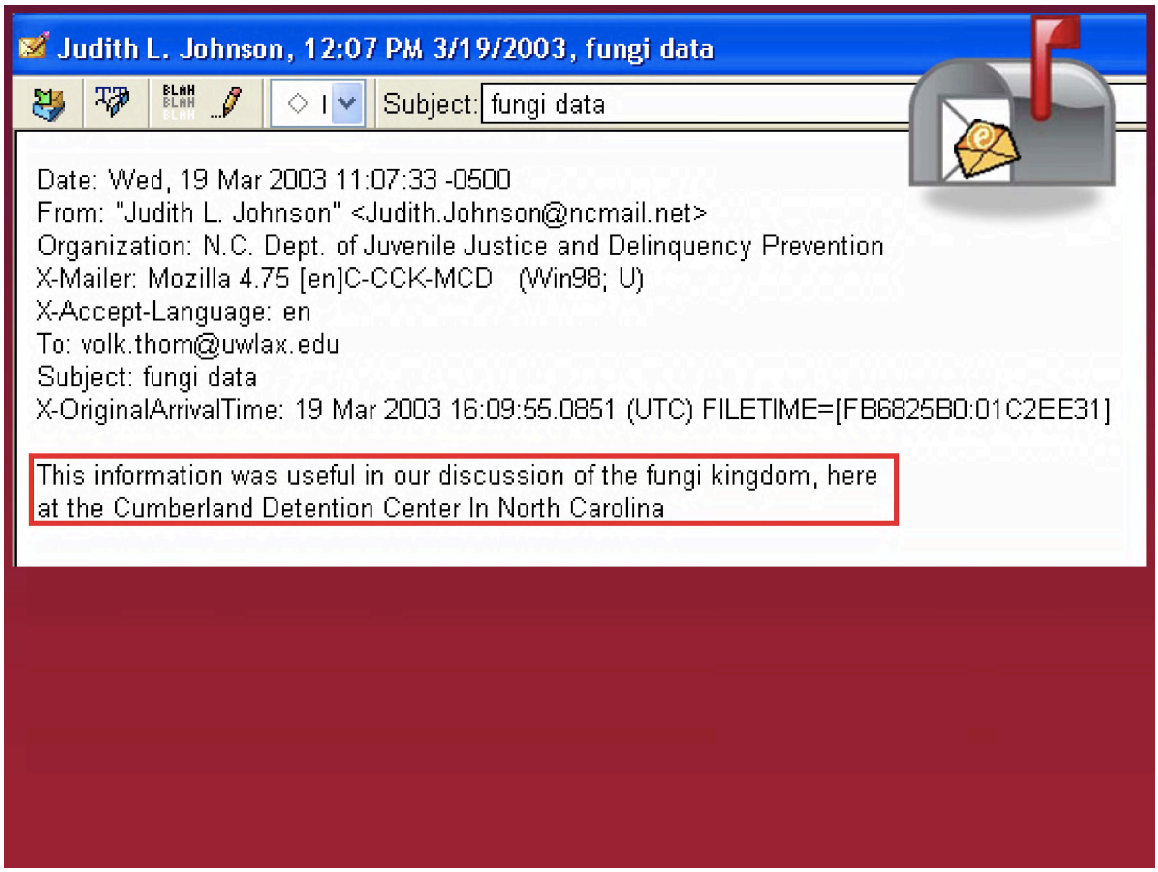
Esquivelse@aol.com, 08:06 PM 3/19/2003, fungi

Subject: fungi

From: Esquivelse@aol.com
Date: Wed, 19 Mar 2003 19:06:31 EST
Subject: fungi
To: volk.thom@uwlax.edu
X-Mailer: 7.0 for Windows sub 10516
X-OriginalArrivalTime: 20 Mar 2003 00:06:50.0542 (UTC) FILETIME=[9B177CE0:01C2EE74]

Do you know more about fungi


Naveed Davoodian, 11:24 AM 4/19/2005, Dear Mr. Volk
Subject: Dear Mr. Volk
Date: Tue, 19 Apr 2005
From: "Naveed Davoodian" <naveeddavoodian@yahoo.com>
Subject: Dear Mr. Volk
To: <volk.thom@uwlax.edu>
Dear Mr. Volk,
I am an 18 year old mycology enthusiast and I am in the midst of a serious fungal dilemma. I cannot identify the following 3 species of fungi! I was trekking through the woods near a swamp about 3 weeks ago (i live in central Florida) and I came across these specimens. I snapped photos and went home to identify them, but my field guide did not suffice. I then surfed the internet until my eyes began to hurt, but still no sign of these fungi.
can you help me?!
photographs of the 3 species are attached.

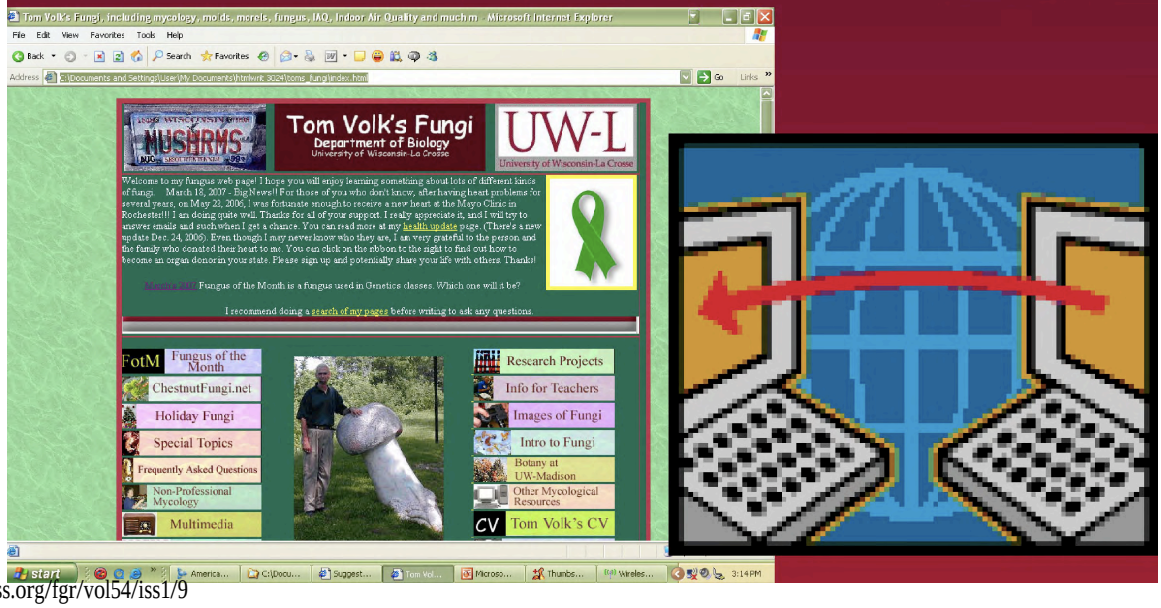

Judith L. Johnson, 12:07 PM 3/19/2003, fungi data
Subject: fungi data
Date: Wed, 19 Mar 2003 11:07:33 -0500
From: "Judith L. Johnson" <Judith.Johnson@ncmail.net>
Organization: N.C. Dept. of Juvenile Justice and Delinquency Prevention
X-Mailer: Mozilla 4.75 [en]C-CCK-MCD (Win98; U)
X-Accept-Language: en
To: volk.thom@uwlax.edu
Subject: fungi data
X-OriginalArrivalTime: 19 Mar 2003 16:09:55.0851 (UTC) FILETIME=[FB6825B0:01C2EE31]
This information was useful in our discussion of the fungi kingdom, here at the Cumberland Detention Center In North Carolina

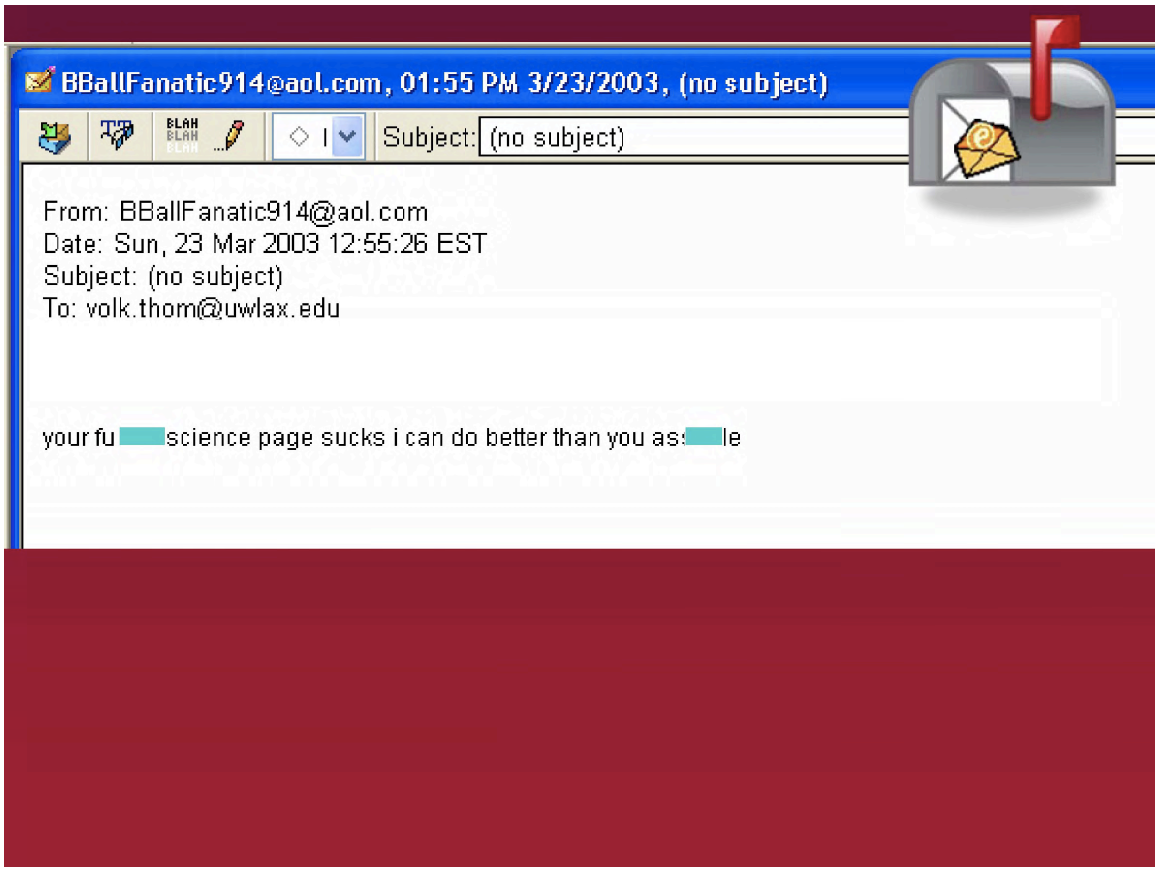
Educational activity - Birthday Internet Assignment

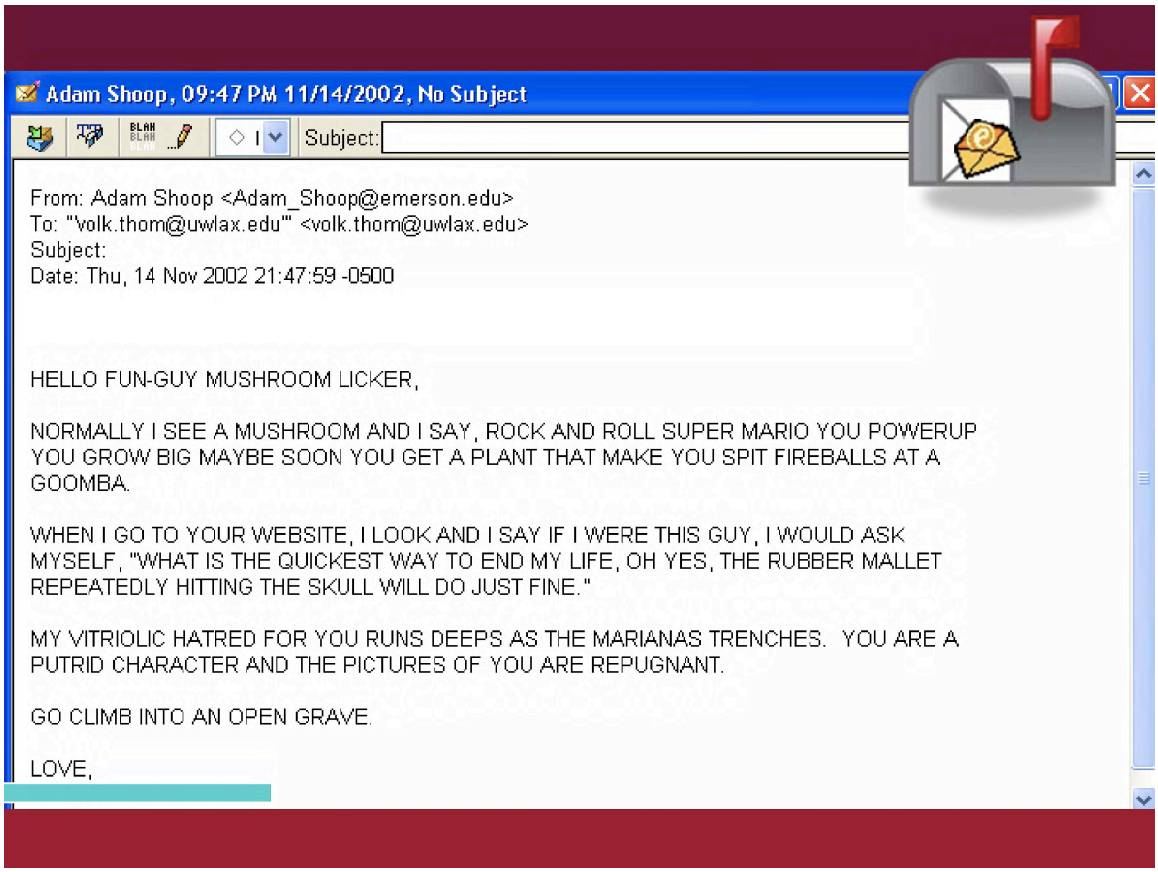
- To be sure students have read my web pages and know what's available on them, I give my students this assignment. Judging by the responses I get, students seem to really like doing it:
- Please visit TomVolkFungi.net Look around and see what you can find! There are many pictures and descriptions of the fungi we'll be talking about in lecture.
- For your assignment, determine the "Fungus of the Month" for YOUR birthday month. In all cases you will have several choices for that month, since I started the Fungus of the Month in January of 1997.
- Then after reading about the fungus of the month for your birthday month send me an email with the following things in it:
- Body of email
  1. Your name 2. Month of your birthday
  3. Fungus of the month for your birthday month
  4. What is the food source for that species? Is the fungus a saprophyte, parasite or mutualist?
  5. To which phylum does that fungus belong ?
  6. Write a few sentences about why that fungus is interesting.

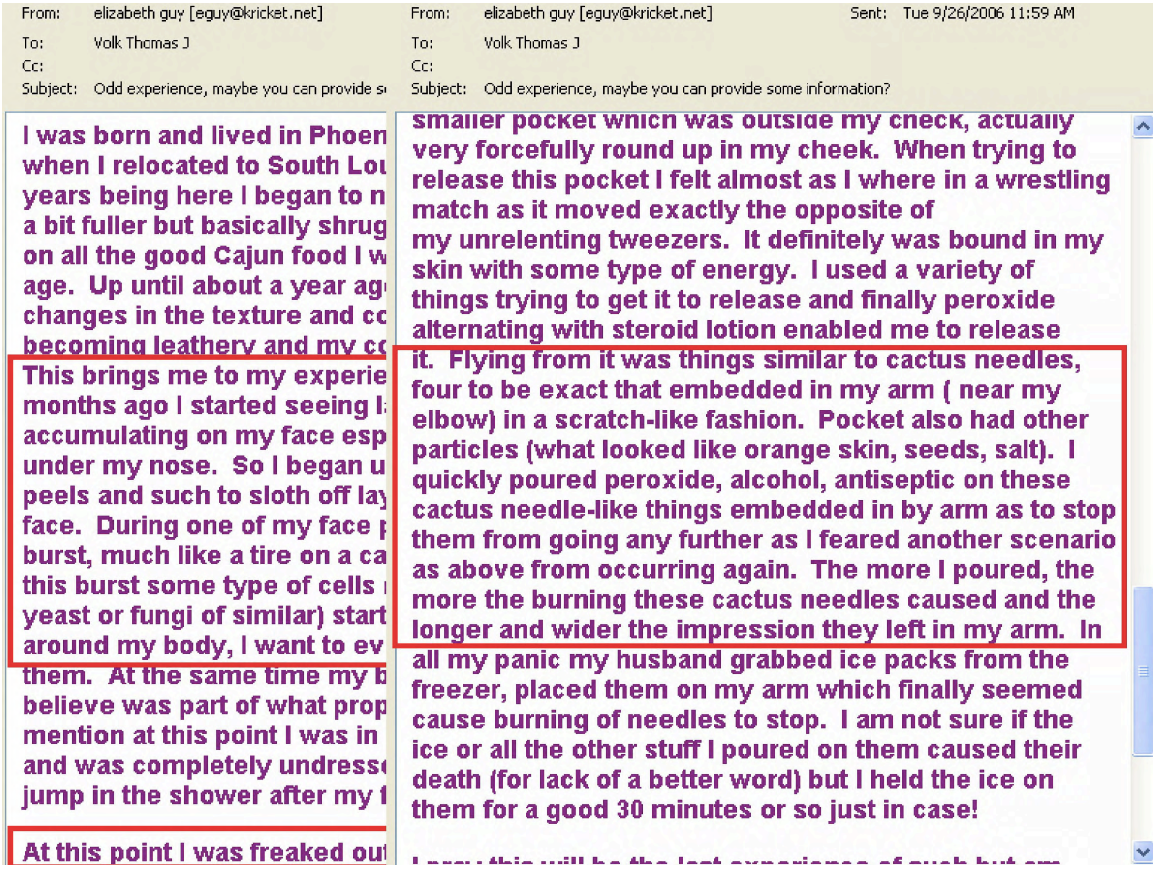
Why have web pages online?

- Some days I wonder…




BBallFanatic914@aol.com, 01:55 PM 3/23/2003, (no subject)
Subject: (no subject)
From: BBallFanatic914@aol.com
Date: Sun, 23 Mar 2003 12:55:26 EST
Subject: (no subject)
To: volk.thom@uwlax.edu
your fu science page sucks i can do better than you as: le


Adam Shoop, 09:47 PM 11/14/2002, No Subject
Subject:
From: Adam Shoop <Adam_Shoop@emerson.edu>
To: "volk.thom@uwlax.edu'" <volk.thom@uwlax.edu>
Subject:
Date: Thu, 14 Nov 2002 21:47:59 -0500
HELLO FUN-GUY MUSHROOM LICKER,
NORMALLY I SEE A MUSHROOM AND I SAY, ROCK AND ROLL SUPER MARIO YOU POWERUP YOU GROW BIG MAYBE SOON YOU GET A PLANT THAT MAKE YOU SPIT FIREBALLS AT A GOOMBA.
WHEN I GO TO YOUR WEBSITE, I LOOK AND I SAY IF I WERE THIS GUY, I WOULD ASK MYSELF, "WHAT IS THE QUICKEST WAY TO END MY LIFE, OH YES, THE RUBBER MALLET REPEATEDLY HITTING THE SKULL WILL DO JUST FINE."
MY VITRIOLIC HATRED FOR YOU RUNS DEEPS AS THE MARIANAS TRENCHES. YOU ARE A PUTRID CHARACTER AND THE PICTURES OF YOU ARE REPUGNANT.
GO CLIMB INTO AN OPEN GRAVE.
LOVE,



From: elizabeth guy [eguy@kricket.net]
To: Volk Thomas J
Cc:
Subject: Odd experience, maybe you can provide s

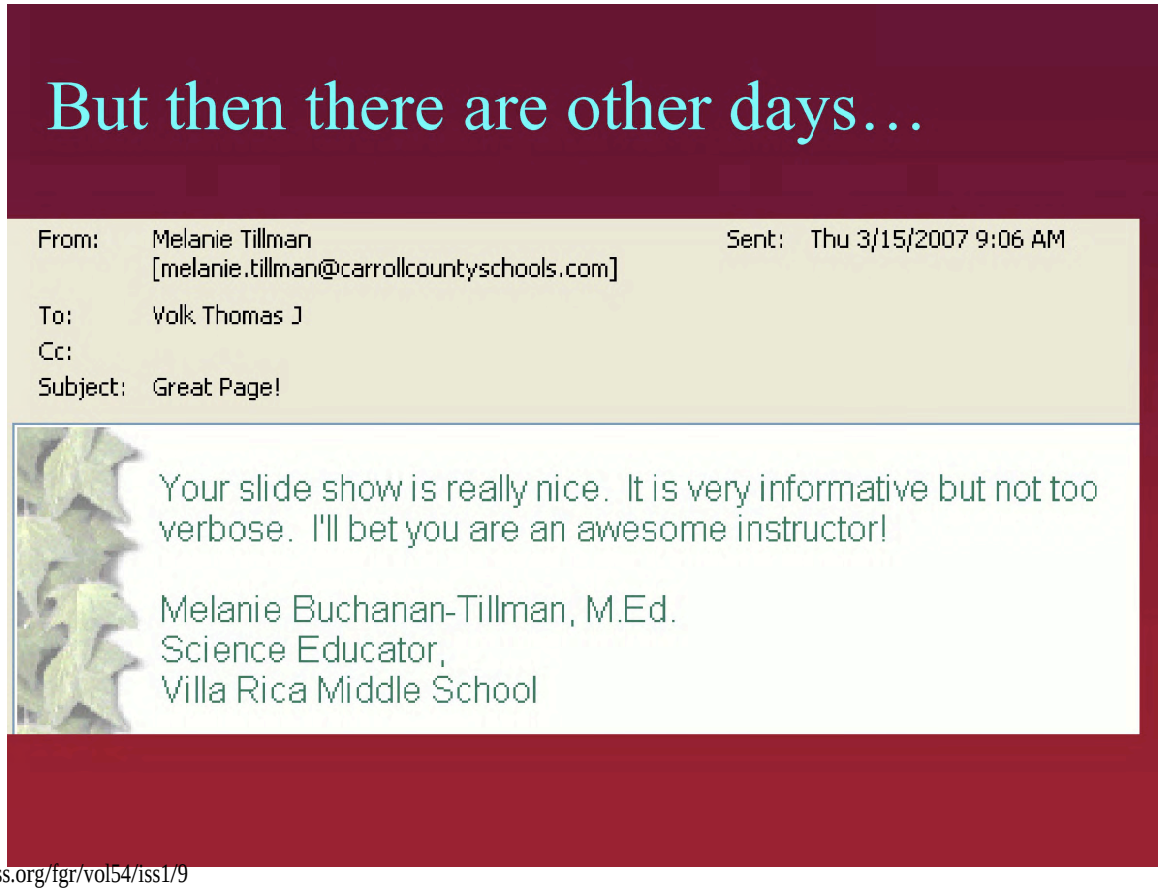
I was born and lived in Phoen
when I relocated to South Lou
years being here I began to n
a bit fuller but basically shrug
on all the good Cajun food I w
age. Up until about a year ag
changes in the texture and co
becoming leathery and my co
This brings me to my experie
months ago I started seeing l
accumulating on my face esp
under my nose. So I began u
peels and such to sloth off lay
face. During one of my face p
burst, much like a tire on a ca
this burst some type of cells
yeast or fungi of similar) start
around my body, I want to ev
them. At the same time my b
believe was part of what prop
mention at this point I was in
and was completely undresse
jump in the shower after my f

At this point I was freaked out

From: elizabeth guy [eguy@kricket.net] Sent: Tue 9/26/2006 11:59 AM
To: Volk Thomas J
Cc:
Subject: Odd experience, maybe you can provide some information?

smaller pocket which was outside my cheek, actually very forcefully round up in my cheek. When trying to release this pocket I felt almost as I where in a wrestling match as it moved exactly the opposite of my unrelenting tweezers. It definitely was bound in my skin with some type of energy. I used a variety of things trying to get it to release and finally peroxide alternating with steroid lotion enabled me to release it. Flying from it was things similar to cactus needles, four to be exact that embedded in my arm (near my elbow) in a scratch-like fashion. Pocket also had other particles (what looked like orange skin, seeds, salt). I quickly poured peroxide, alcohol, antiseptic on these cactus needle-like things embedded in by arm as to stop them from going any further as I feared another scenario as above from occurring again. The more I poured, the more the burning these cactus needles caused and the longer and wider the impression they left in my arm. In all my panic my husband grabbed ice packs from the freezer, placed them on my arm which finally seemed cause burning of needles to stop. I am not sure if the ice or all the other stuff I poured on them caused their death (for lack of a better word) but I held the ice on them for a good 30 minutes or so just in case!

Sorry this will be the last experience of such but am



SeashellPM@aol.com, 08:49 PM 11/5/2002, help! (please)

Subject: help! (please)

SeashellPM@aol.com, 05:52 PM 11/14/2002, Re: help! (please)

Subject: Re: help! (please)

Date: Thu, 14 Nov 2002 17:52:15 -0500
From: SeashellPM@aol.com
To: volk.thom@uwlax.edu
Subject: Re: help! (please)

Dr. Volk: thank you so much!! Wow! there's no one I'd like to talk to more (except maybe Paul McCartney ☺)!

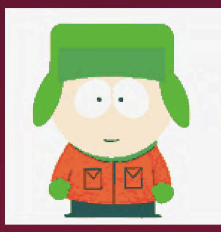
Sorry for keeping you waiting so long, I've been having some trouble with the internet…

I live in Phoenix, AZ and, sadly, do not see many mushrooms here. Occasionally several will crop up in the park after it rains, but my resident city is not noted for its humidity. Thus, I do most of my mushroom sighting in textbooks and field guides. However, I keep a very optimistic view of mushrooms, hoping that someday I will be able to see live specimens with more regularity.

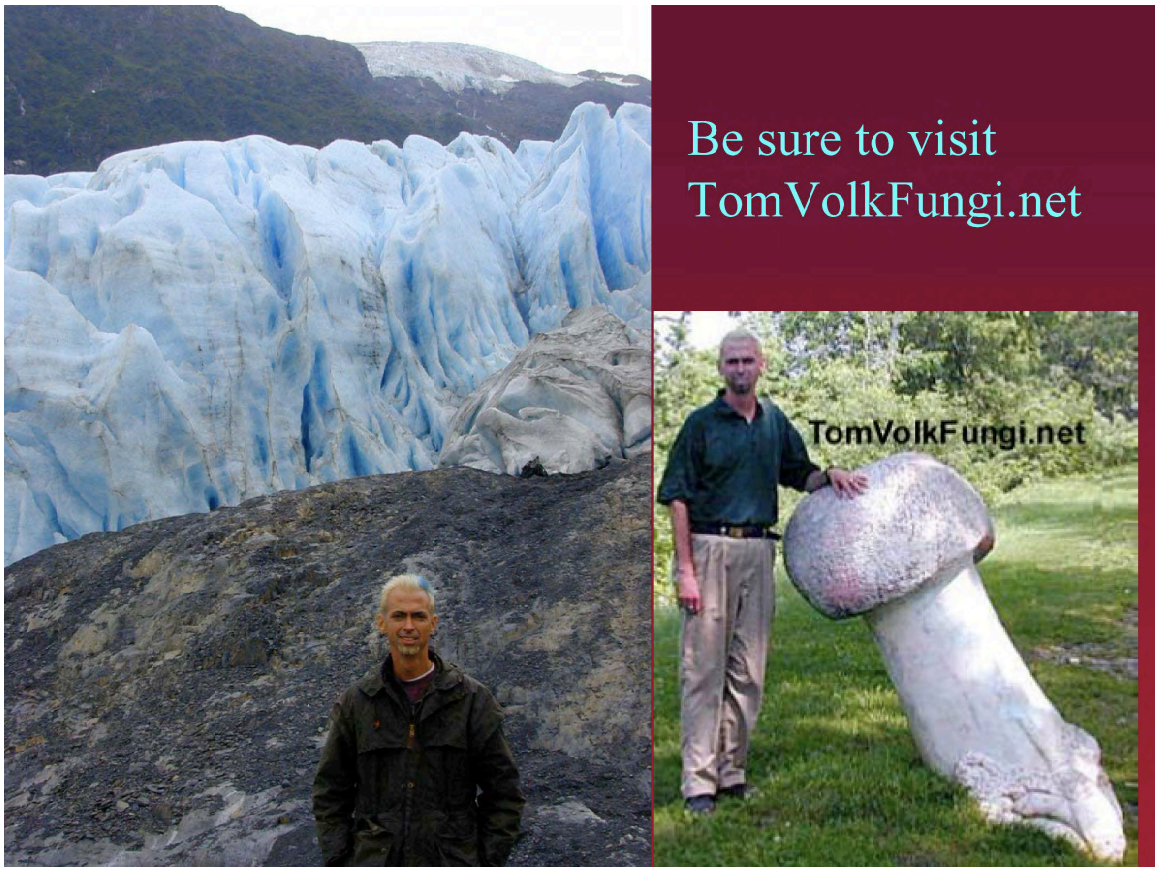
Again, thank you very much! It may be a little while before I can send you my questions, because I have to find out what things I don't know…

~Jennifer

We've learned something today



- Don't be afraid for jungle phonetics.
- The internet can be a powerful tool for education
- Making pages fun and relevant can lead people to visit over and over again.
- If you want to be the co-author of a Fungus of the Month, contact me!



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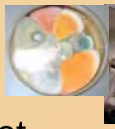
DOI: 10.4148/1941-4765.1103

Teaching Medical Mycology : Luring students into learning about fungi

Thomas J. Volk

Department of Biology, University of Wisconsin-La Crosse, La Crosse WI 54601 volk.thom@uwlax.edu TomVolkFungi.net



243

Abstract

This presentation is the story of a successful Medical Mycology course at the University of Wisconsin-La Crosse (about 8500 students), where enrollment one semester has peaked at 38 students. I started with an enrollment of 13 students in Spring 1997; there have been 250 students in the past 11 years. Through word of mouth and course "advertising," enrollments have steadily increased, especially among microbiology majors and among pre-med students, when they realize they will likely receive little training in mycology in medical school. Calling the course "Medical Mycology" is also a marketing tool to lure students into learning about mycology.

Medical Mycology at the University of Wisconsin-La Crosse

Although fungi are increasing in importance as more patients become immunocompromised and acquire fungal infections, Medical Mycology is not yet a common course in undergraduate or graduate programs, with fewer than 40 full-fledged Medical Mycology courses currently taught in North America, according to Tex Beneke, Al Rogers, and Star Publishing. However, Medical Mycology can be a successful and popular course if taught and "marketed" to students in interesting ways.

The University of Wisconsin-La Crosse is a medium-sized university of about 8500 students, located on the Mississippi River in western Wisconsin. We have about 1200 majors in Biology and Microbiology, with concentrations in Biomedical Sciences, Cell & Molecular Biology, Environmental Sciences, and Aquatic sciences. We also have a Master of Science program with about 60 students. I have eight graduate students in my lab. I teach courses in Medical Mycology, Mycology, Plant-Microbe Interactions, Food & Industrial Mycology, Plant Biology, Organismal Biology, and Latin & Greek for Scientists.

The Lecture: "A study of the yeasts, molds, and actinomycetes that are pathogenic to humans and other animals." Although the prerequisites are Mycology or Intro Microbiology, I assume students have no previous knowledge of fungal biology. My course is somewhat different than most medical mycology courses, since I teach it mostly from the fungus' point of view. Of course, we also discuss the symptoms and treatments for each fungal disease, as well as mycetismus and mycotoxicosis. See the syllabus on the right of this poster.

My background and training is primarily in wood decay fungi, morels, and more general mycology. When I started teaching this course at UW- La Crosse in 1997, I had not previously had a medical mycology course or taught one. I was lucky that my friend, John Rippon, gave me most of his collection of teaching slides (about 2500), which I have scanned for computer use. He also gave me his class notes and handouts, for which I am very grateful. All of my class presentations are now done in PowerPoint, with modifications and (sometimes major) updates every year. See syllabus on the right. The three lecture exams consist of mostly essay questions, requiring students to integrate their knowledge, rather than just memorization.

The Laboratory is the crux of the course. The lab emphasis is on laboratory techniques for isolation and identification of pathogenic fungi. Besides studying many pre-prepared slides, each student makes a permanent reference slide collection of approximately 45 species of pathogenic and "contaminant" fungi, mostly deuteromycetes, using PVLG (polyvinyl-lactic acid-glycerine) to make permanent mounts of slide cultures or tape mounts. Students learn the techniques and skills necessary to identify nearly any deuteromycete or yeast, a very important and sought-after job skill. Students also isolate fungi into pure culture from their environment and are required to identify three of these "unknown" species over the course of the semester. The two lab exams consist of 15-18 set-ups (typically one or more slides, drawings, photographs, books, biochemical tests, and/or posters) with 3-6 questions at each station. These questions tend to be more objective than the lecture exams.

Poster presented at the annual conference of the National Association of Biology Teachers, Oct. 19-22, 2000, Minneapolis, MN

Top ten reasons to take Medical Mycology BIO 413/513 this Spring Semester

10. In this age of immunocompromised people (AIDS, steroid therapy, chemotherapy and environmental pollutants) fungi are becoming ever more important as pathogens of humans and other animals.

9. If you're planning to go into the medical field you won't get many (if any) lectures on mycology in professional schools. Medical schools have in the past been impressed that applicants have take medical mycology here. This course will give you a leg up on your medical school colleagues. It is strongly recommended for the Biology and Microbiology majors, especially in the biomedical concentration.

8. According to Star Publishing (via Tex Beneke and Al Rogers), there are only 39 (thirty-nine) schools in North America where a course in medical Mycology is offered. This is a great opportunity to take this interesting course.

7. You'll see interesting pictures of interesting people who happen to be infected with fungi.

6. You'll learn about poisonous mushrooms, as well as deadly mycotoxins in food.

5. Each student will make a reference slide collection of all the fungal pathogens we study. You can take this collection with you and treasure it forever (maybe even longer). It's actually something that your co-workers will covet.

4. Dealing with medical fungi in a clinical setting is a practical skill that will increase your job possibilities. Companies and medical professionals want workers with training in mycology.

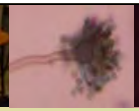
3. We hold a poster session where you and your friends can make and look at posters and eat interesting foods.

2. You'll get to see lots of Skin Flicks (and learn how to cure them)

1. Everyone's doing it. C'mon you know you want to…

Lecture meets 8:50-9:45 MW. There is a choice of two labs: 8:50-10:50 F or 11-1 F.
For more info see Tom Volk in 3024 Cowley Hall or TomVolkFungi.net

Taking pictures with Nikon CoolPix 995 through the lens of the microscope


Aspergillus, taken with digital camera through the microscope lens

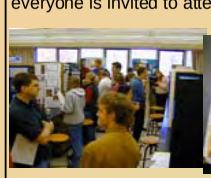
Cryptococcus, taken with digital camera through the microscope lens

Poster sessions

It is important for students to know how to research a topic outside the sphere of what they learn in class. In many courses the professor assigns a term paper covering a particular topic. A student researches the material and writes a more or less coherent essay on the topic. However, the other students in the class learn nothing about any topic except their own. In addition, term papers can be tedious and time-consuming for the professor to grade, especially since these generally are due at the end of the year when everything else is becoming busy.

One alternative is to hold a poster session, an idea I got from Mike Tansey of Indiana University, who has had his students make posters for their mycology lab for years. I have taken his idea one step further and hold an actual poster session

We have a formal poster session, much like the one you're attending right now, but with better food, since students can bring treats, often related to their topics. Each student presents a poster on a self-chosen topic, similar to my fungal web pages at http://TomVolkFungi.net, where many medically important fungi have been featured. The poster session is a great learning tool for mycology courses. Students can learn more in depth about a particular topic that interests them, and in addition they can learn something about other students' chosen topics. The poster sessions are advertised to students and faculty in the department and everyone is invited to attend.



Assignment for poster session:

Your poster should include information on a medical mycology topic of your choice. It can be a disease, a treatment, an organ that's affected in different ways. Use your imagination. You should clear the topic with me in advance; try to pick something not covered in class. It's a good idea to pick something that might help in your future career

Some advantages of the Poster session:

- Students experience researching a topic in depth
- Students get to show their creativity in ways other than writing
- Students gain experience making a poster
- Students gain experience presenting a poster to an audience
- Students get the experience of participating in a poster session in a relatively low pressure situation, i.e. not at their first scientific meeting
- Students learn about other topics not assigned to or chosen by them
- Posters are easier to grade than term papers
- Posters are available for use in the course and in other courses in following semesters (and at scientific meetings and forays)
- Visiting students and staff not in the class can learn something about mycology and might be influenced to take mycology someday
- Mycology is promoted in the department and the university

Volk, Thomas J. 2001. Poster Sessions as teaching and learning tools in Mycology courses. MICOLOGIA APLICADA INTERNATIONAL 13(1): 45-49

Syllabus: Medical Mycology BIO 413/513

Dr. Tom Volk, 3024 Cowley Hall 785-6972
Lecture meets MW 8:50-9:45, Labs 8:50-10:50 F or 11-1 F.

1	Introduction	
	Classification systems	
	LAB 1	General Mycology
2	Fungal Life Cycles, deuteromycetes	
	Lab procedures, contamination	
	LAB 2	General Mycology
3	Fungal physiology and pathogenesis	
	Lab Diagnosis /Antifungal therapy	
	LAB 3	Common Fungal Contaminants
4	***EXAM I** General Mycology*	
	Superficial Mycoses	
	LAB 4	Common Fungal Contaminants
5	Dermatophytes	
	Dermatophytes	
	LAB 5	Superficial Mycoses and Dermatophytes
6	Chromoblastomycosis & Mycetoma	
	Phaeohyphomycosis,Sporotrichosis	
	LAB 6	Dermatophytes
7	Pseudallescheriasis	
	Mushroom Poisoning	
	LAB 7	***LAB EXAM I***
	SPRING BREAK	
8	Mycotoxicosis, mycoallergies	
	Lab identification of yeasts	
	LAB 8	Subcutaneous fungal infections
9	***EXAM II***	
	Candidiasis	
	LAB 9	Pathogenic yeasts
10	Candidiasis	
	Cryptococcosis & other yeast inf	
	LAB 10	Pathogenic yeasts
11	More Yeast infections	
	Intro to systemic mycoses	
	LAB 11	Poster session
12	Histoplasmosis	
	Blastomycosis	
	LAB 12	Fungi of deep mycoses
13	Coccidioidomycosis & Paracoccidioidomycosis	
	Opportunistic infections	
	LAB 13	Fungi of deep mycoses
14	Opportunistic inf., + Actinomycetes	
	Review	
	LAB 14 LAB EXAM	***LAB EXAM II***
	Final Exam (Exam III)	**Partially comprehensive**

All topics, exams, and schedules subject to change.

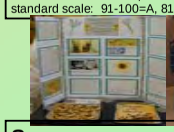
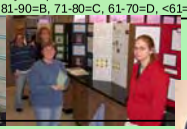
Text: Kwon-Chung, K.J. & John E. Bennett, 1992. Medical Mycology. Philadelphia & London: Lea & Febiger

Lab: Beneke, Everett S & Alvin L. Rogers. 1996. Medical Mycology and Human Mycoses. Belmont CA: Star Publishing -- also Dr. Allen Nelson's lab manual (modified by T.Volk) handed out in class.

Grading:

Lecture Exams Exam I	70 pts.
Exam II	130 pts.
Exam III	130 pts.
Lab Exams 2 @ 120	240 pts.
Slide Collection	30 pts.
Quizzes, internet assignments, etc.	~130 pts.
Unknowns 15 pts each—three filamentous and 1 yeast	60 pts
Poster on an interesting medical mycology topic	50 pts.
Total	~800 pts.

If you miss a lecture exam, the only way to make it up will be as an oral exam. There will be no make-up exams for missed lab exams—it is impossible to reconstruct a lab exam or leave it up for more than the time allotted. All exams are partially comprehensive. Grades are assigned on a standard scale: 91-100=A, 81-90=B, 71-80=C, 61-70=D, <61=F.

Summary

It's a lot of work, especially with the lab, but Medical Mycology is a very worthwhile course to teach, especially to undergraduates and to Pre-med and other biomedical students who will likely encounter mycology very little in their post-baccalaureate training.

Published by New Prairie Press 2017

Fungal Gene and Protein Information at the *Saccharomyces* Genome Database (SGD) and *Candida* Genome Database (CGD)

Maria C. Costanzo
Department of Genetics • Stanford University School of Medicine
24th Fungal Genetics Conference
March 21, 2007

Overview

- What types of information are available at SGD and CGD?
- How do I find and download information?
- How do I submit information?

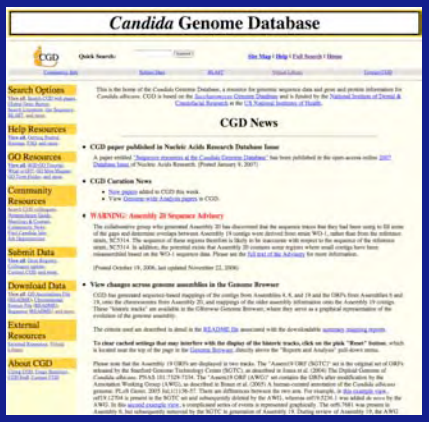
yeast-curator@genome.stanford.edu

candida-curator@genome.stanford.edu

https://newprairiepress.org/fgr/vol54/iss1/9
DOI: 10.4148/1941-4765.1103

SGD and CGD home pages

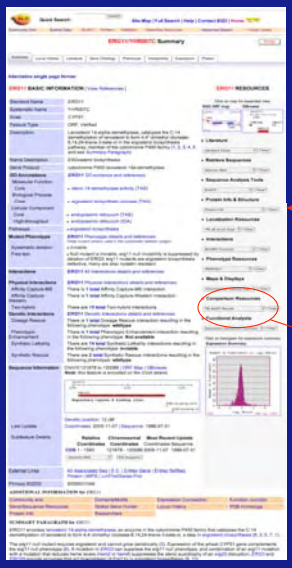
http://www.yeastgenome.org



http://www.candidagenome.org

Locus Summary page

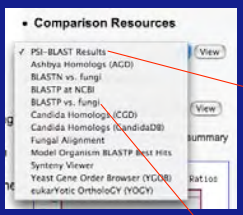
SGD



CGD

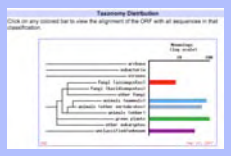
Potential orthologs in other fungi

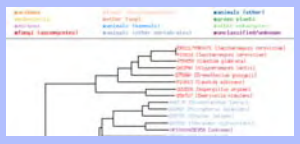
SGD

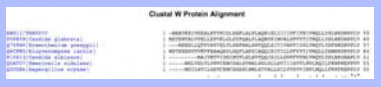


Fungal BLAST

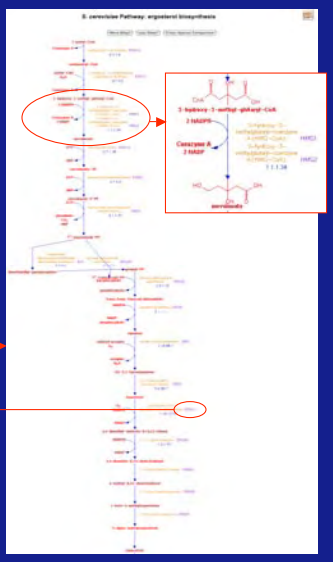
PSI-BLAST







Graphical displays of biochemical pathways



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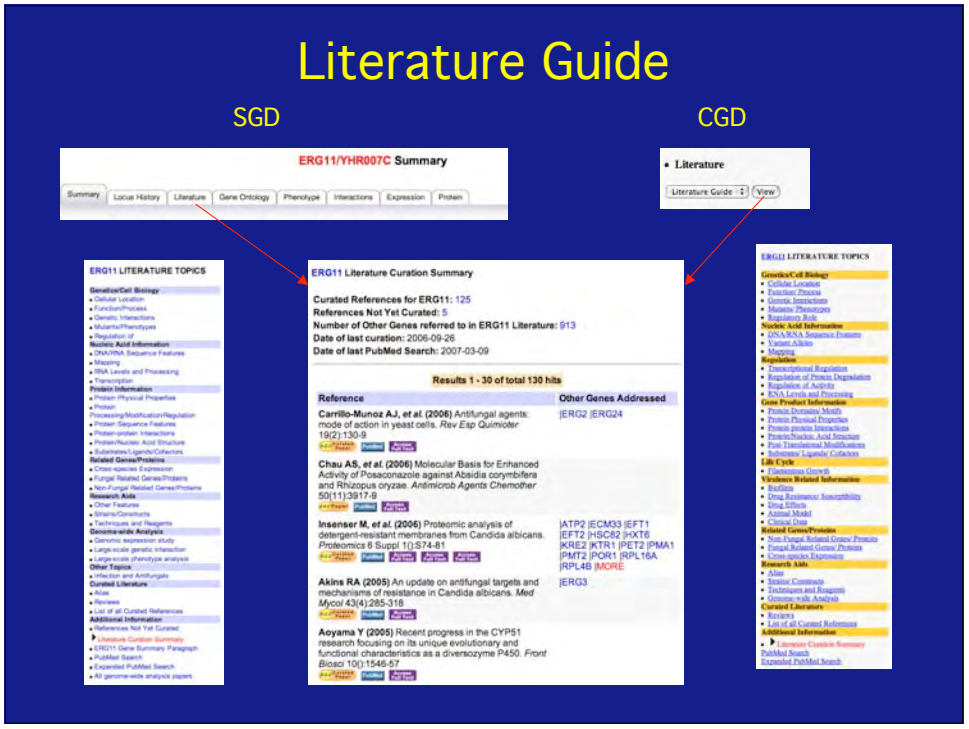
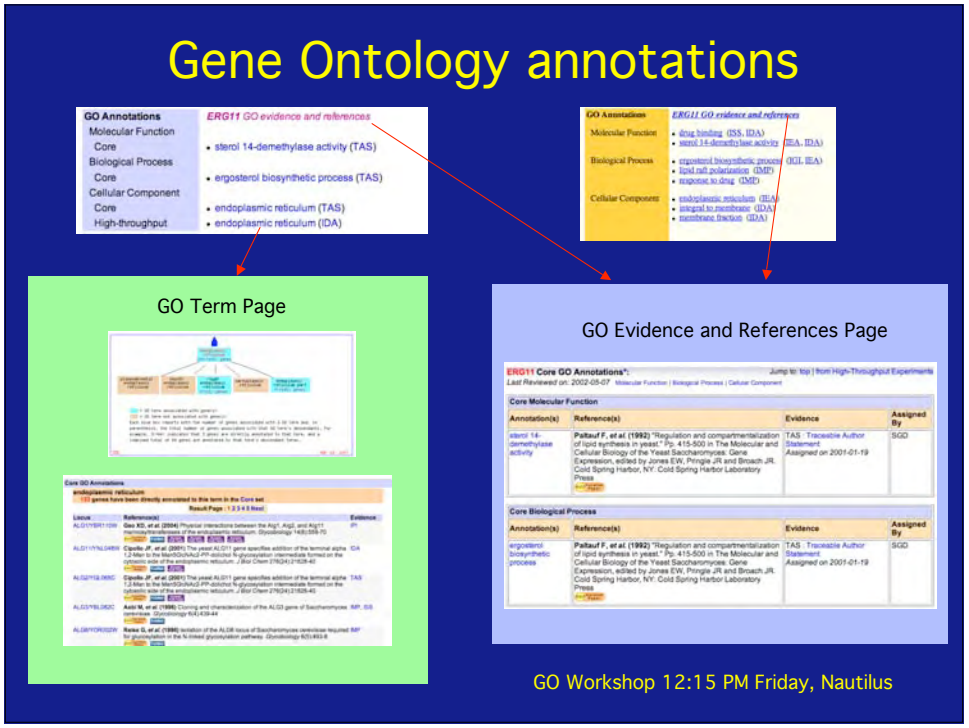
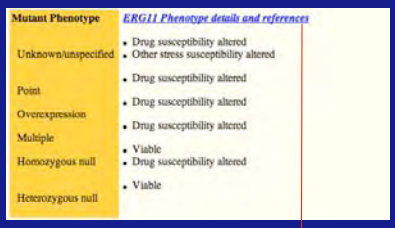

Literature Guide
SGD
CGD
ERG11/YHR007C Summary
Literature
ERG11 LITERATURE TOPICS
ERG11 Literature Curation Summary
Curated References for ERG11: 125
References Not Yet Curated: 5
Number of Other Genes referred to in ERG11 Literature: 913
Date of last curation: 2006-09-26
Date of last PubMed Search: 2007-03-09
Results 1 - 30 of total 130 hits
Reference
Other Genes Addressed
Carrillo-Munoz AJ, et al. (2006) Antifungal agents: mode of action in yeast cells. Rev Esp Quimioter 19(2):130-9
|ERG2 |ERG24
Chau AS, et al. (2006) Molecular Basis for Enhanced Activity of Posaconazole against Absidia corymbifera and Rhizopus oryzae. Antimicrob Agents Chemother 50(11):3917-9
Insenser M, et al. (2006) Proteomic analysis of detergent-resistant membranes from Candida albicans. Proteomics 6 Suppl 1():S74-81
|ATP2 |ECM33 |EFT1 |EFT2 |HSC82 |HXT6 |KRE2 |KTR1 |PET2 |PMA1 |PMT2 |POR1 |RPL16A |RPL4B |MORE
Akins RA (2005) An update on antifungal targets and mechanisms of resistance in Candida albicans. Med Mycol 43(4):285-318
|ERG3
Aoyama Y (2005) Recent progress in the CYP51 research focusing on its unique evolutionary and functional characteristics as a diversozyme P450. Front Biosci 10():1546-57

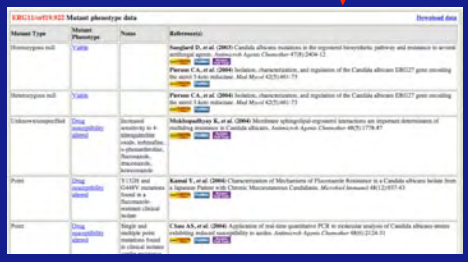

Gene Ontology annotations
GO Annotations
ERG11 GO evidence and references
Molecular Function
Core
sterol 14-demethylase activity (TAS)
Biological Process
Core
ergosterol biosynthetic process (TAS)
Cellular Component
Core
endoplasmic reticulum (TAS)
High-throughput
endoplasmic reticulum (IDA)
GO Term Page
GO Evidence and References Page
ERG11 Core GO Annotations*:
Core Molecular Function
Core Biological Process
GO Workshop 12:15 PM Friday, Nautilus

Mutant phenotype data

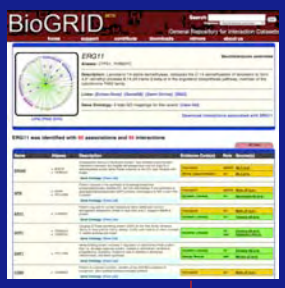
SGD

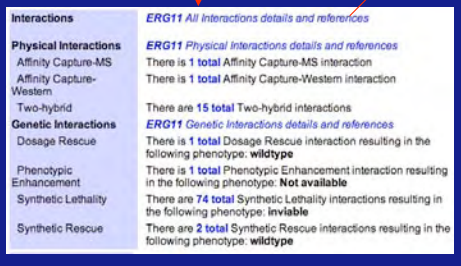
CGD





Genetic and physical interaction data (SGD)





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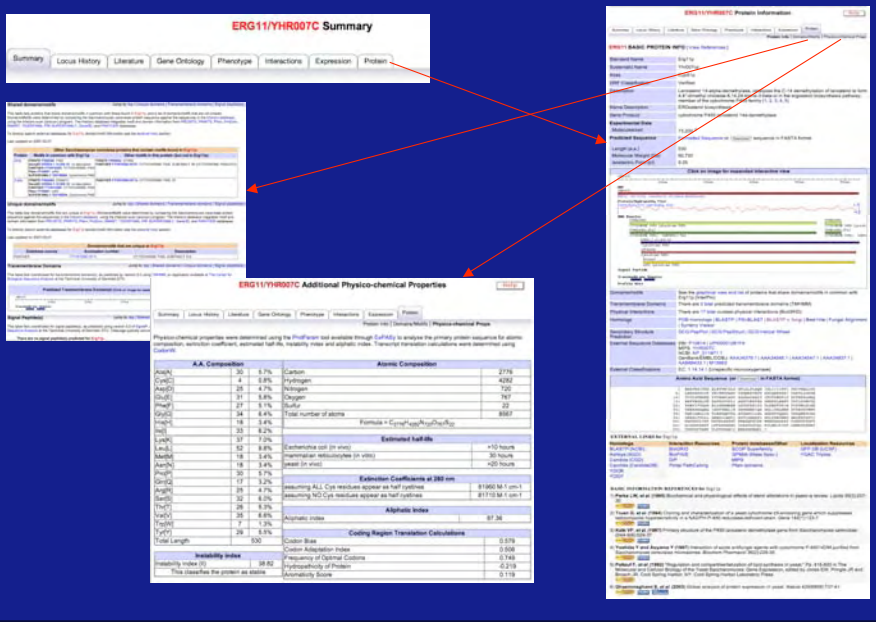
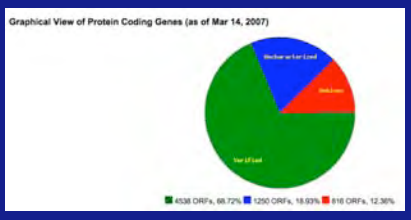
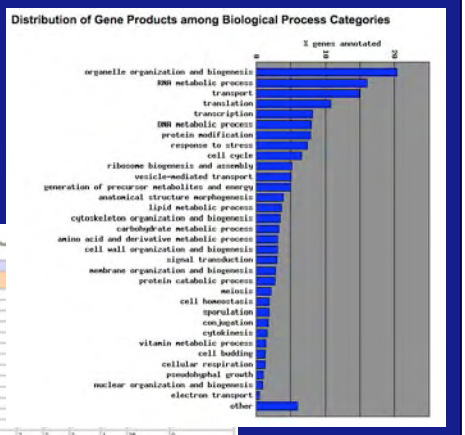
DOI: 10.4148/1941-4765.1103

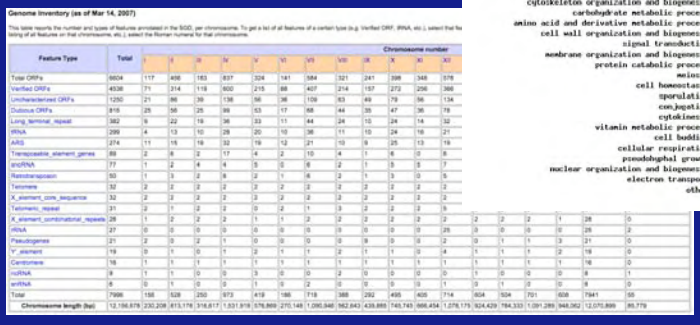
Protein information (SGD)



Getting an overview: SGD's Genome Snapshot

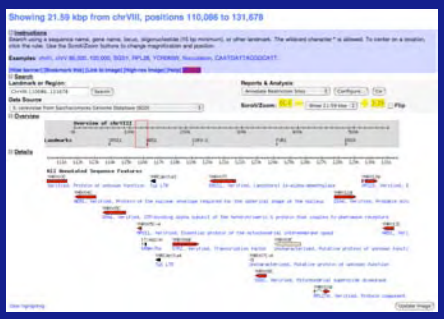






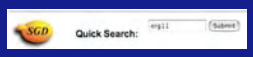
Genome navigation: GBrowse

SGD



CGD

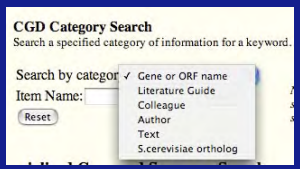
Finding information



- gene names
- gene products
- GO terms
- Colleagues
- authors
- biochemical pathways
- descriptions
- PubMed ID
- GO ID
- external ID

- gene names
- gene products
- GO terms
- Colleagues
- authors

SGD Advanced Search



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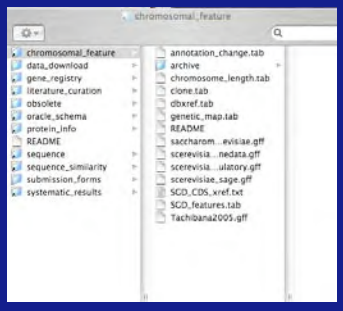
Downloading information

SGD



CGD

SGD's Batch Download tool

Submitting information

Colleague information

Gene Registries

SGD community wiki

Contact us anytime!

yeast-curator@genome.stanford.edu

candida-curator@genome.stanford.edu

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Best Practices & Trouble Spots - Round Table

Amy J. Reese
Cedar Crest College

Amount taught on fungi

- Time given to fungal biology/genetics:
  - Entire courses offered yearly
  - Courses offered periodically
  - A few days to a week in a semester
  - One day/lab in a semester
  - Other

Break into groups…

- Gather with others who teach:
  - Group A: Entire courses
  - Group B: A few days to a week
  - Group C: One day/lab in a semester

Within your group address:

- **Best practices:**
  - What approaches, assignments or labs work well?
  - What do students enjoy & learn from the most?
- **Trouble spots:**
  - What topics, assignments, or labs are not ideal?
  - What do students find boring & not learn?
- **What the other groups could help with:**
  - If you teach a full course, what introductory material do you wish students had already been exposed to?
  - If you only get a few days, what suggestions of highlights could you use from those who cover more?

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Summary……

- Nomenclature: analogy w/best friend's name (not most interesting thing about them, but can use it on "My Space")
- Pathways: use as menonic
- Mycology for non-majors: title ("The Fungal Jungle"), book (Magical Mushrooms etc)
- Expts for non-majors: fungal Ames test w/ substances students provide (deep-fat fryer)
- Practical: start w/ unknown sequence-annotation; no "wrong" answers

Summary 2……

- What do students need to retain from intro courses? Experimental design, more understanding of plant biology needed for fungal biology
- Use of unsolved problems
- Student review papers, how improve quality? Websites as alternative to papers (see upcoming article in GENETICS about "secret paper")

Deleting *Aspergillus nidulans* checkpoint regulators in an undergraduate molecular genetics course

Steve James Department of Biology, Gettysburg College, Gettysburg PA
sjames@gettysburg.edu

Biology 351: *Molecular Genetics*

Enrollment: 12 students, mostly junior/senior Biochemistry/Molecular Biology majors

Prerequisites: *Genetics* (Bio 211) and *Cell Biology* (Bio 212).
Core requirements for the Biology and BMB majors

Format: 3 one-hour lectures, one 4-hour lab x 14-weeks

Curriculum: Core requirement for the BMB major, elective for Biology
Fulfills the capstone requirement in Biology and BMB

Laboratory budget: ~ $3500.00

Three advances in technology make it feasible for pairs of students to delete a gene in one semester

1st The *Ku70* knockout eliminates nearly all ectopic integration. Most integration events occur by homologous recombination

Gene Targeting in Aspergillus

B

Flanking DNA	Transformants tested	Histone H1-RFP	% Positive Transformants
2000 bp	60	54	90%
1000 bp	50	46	92%
500 bp	36	32	89%

Copyright © 2006 by the Genetics Society of America
DOI: 10.1534/genetics.105.052563

A Versatile and Efficient Gene-Targeting System for *Aspergillus nidulans*

Tania Nayak,* Edyta Szewczyk,* C. Elizabeth Oakley,* Aysha Osmani,* Leena Ukil,* Sandra L. Murray,† Michael J. Hynes,† Stephen A. Osmani* and Berl R. Oakley*,†

*Department of Molecular Genetics, The Ohio State University, Columbus, Ohio 43210 and †Department of Genetics, The University of Melbourne, Parkville, Victoria 3010, Australia

Manuscript received October 17, 2005
Accepted for publication December 23, 2005

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2nd Double-joint PCR, aka 2-way and 3-way fusion PCR

Deletion & tagging constructs can be produced using PCR only, w/o DNA cloning. Resulting DNAs can be transformed directly

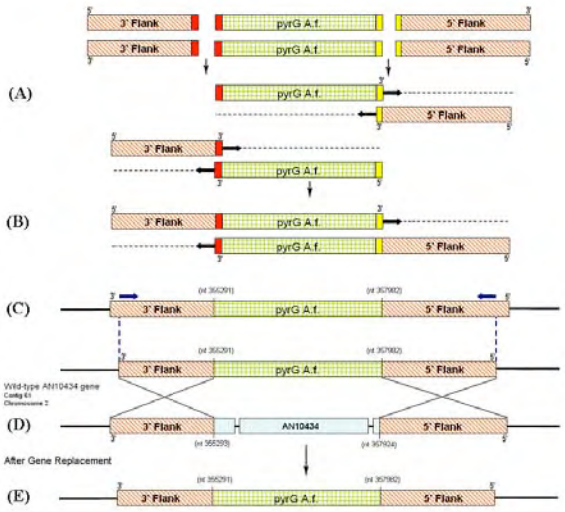


Diagram courtesy of Nick Boire, '07, BMB major

3rd An inexpensive & reliable source of cell-wall degrading enzymes

Vinoflow® FCE winemaking enzyme

100g, ~ $40.00

Use: approximately 3-4 g per experiment

The laboratory project in Molecular Genetics, Fall 2006

Each 2-person team will ---

(1) delete a gene and study the phenotypic consequences

(2) clone the gene to an inducible promoter for overexpression

AN4279: RAD53/Chk2 checkpoint kinase

AN8255: Rad9^{Sc} & *Hu*53BP1-related;
transducer of DNA damage checkpoint signal

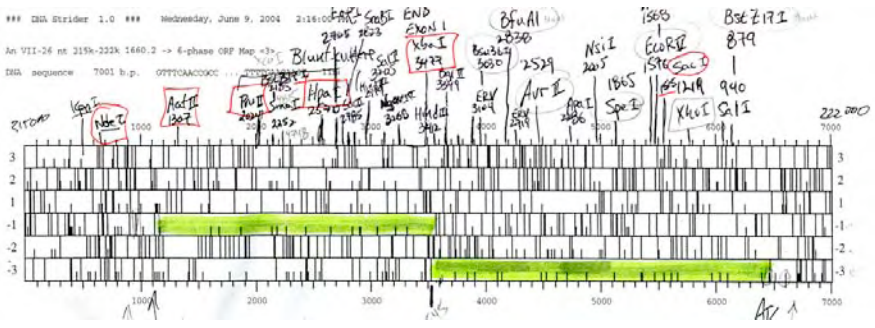
AN6303: RFC1 subunit of the PCNA clamp loader

AN10434: RTT107/ESC4^{Sc} & BRC1^{Sp}-related;
DNA damage tolerance and repair

Week 1: Design gene deletions

The early design phase is a bit messy & can be overwhelming…

….*just as it should* (to stimulate higher-order thinking?)...



https://newprairiepress.org/fgr/vol54/iss1/9
DOI: 10.4148/1941-4765.1103

Assignment for Friday:

1. Locate and mark the START and STOP codons for your gene on the Full Restriction Map.
2. On the Full Restriction Map, locate and mark all of the following restriction enzyme cut sites:

- *Kpn*I - *Sph*I - *Pst*I - *Mlu*I - *Sna*BI - *Bam*HI - *Eco*RI - *Sal*I - *Hind*III - *Xho*I

- *Sty*I - *Avr*II - *Nhe*I - *Xba*I - *Bxt*XI - *Pml*I - *Apa*I - *Sca*I - *Eag*I - *Not*I

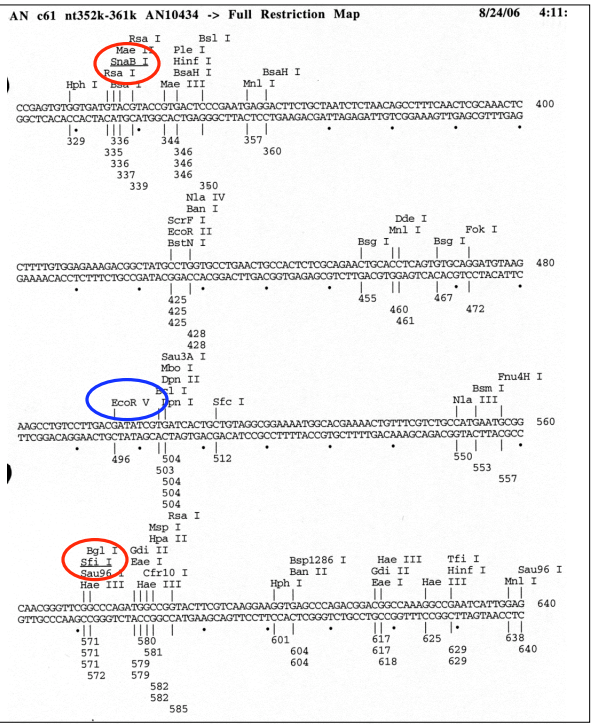
3. On the 6-phase ORF Map, pencil in the position of each of the sites above. Include the nucleotide number along with the enzyme name at each position.
4. Using the *A. nidulans* Database, determine on which chromosome your chosen gene resides and its approximate location. Print out the chromosome map and draw the location of your gene on the map.

Example of a
Full Restriction Map

Shows…

-- dsDNA sequence

-- Restriction Enzyme cut sites



Directory of site usage

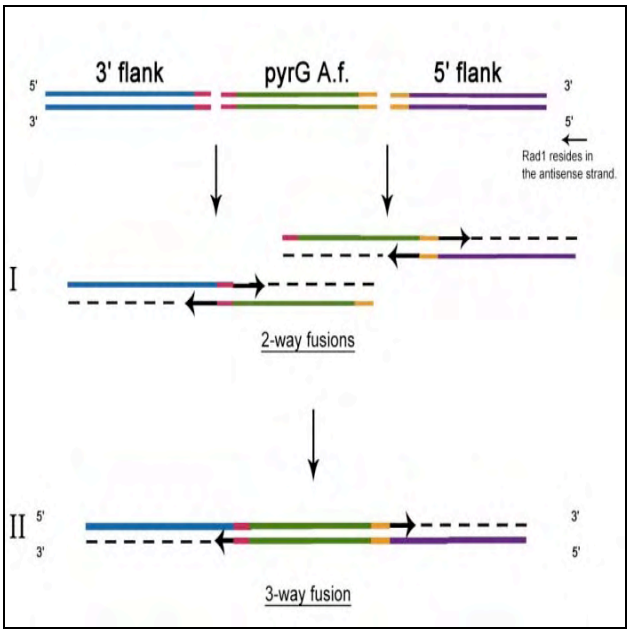
Location of restriction sites

Restriction Endonucleases site usage

Aat II	3	Bsp1286 I	14	Gdi II	8	PflM I	1
Acc65 I	-	BspD I	-	Gsu I	2	Ple I	17
Acc I	3	BspE I	2	Hae I	7	Pme I	1
Aci I	44	BspH I	1	Hae II	9	Pml I	1
Afl II	1	BspM I	3	Hae III	25	PpuM I	3
Afl III	5	BspM II	2	Hga I	17	Pst I	2
Age I	2	Bsr I	17	HgiA I	8	Pvu I	-
Aha II	7	BssH II	3	Hha I	44	Pvu II	2
Alu I	35	Bst1107 I	1	HinC II	8	Rev prim(a)	-
Alw I	13	BstB I	2	HinD III	5	Rev prim(b)	-
AlwN I	4	BstE II	2	Hinf I	38	Rma	21
Apa I	-	BstN I	20	HinP I	44	Rsa I	21
ApaL I	2	BstU I	30	Hpa I	2	Rsr II	3
Apo I	10	BstX I	1	Hpa II	36	Sac I	2
Asc I	-	BstY I	8	Hph I	17	Sac II	-
Ase I	-	Bsu36 I	2	Kas I	2	Sal I	1
Asp718	-	Cfr10 I	8	Kpn I	-	Sau3A I	32
Ava I	9	Cla I	-	Mae I	21	Sau96 I	23
Ava II	15	Dde I	36	Mae II	19	Sca I	2
Avr II	3	Dpn I	32	Mae III	25	ScrF I	37
Bal I	3	Dpn II	32	Mbo I	32	Seq prim(a)	-
BamH I	1	Dra I	2	Mbo II	50	Seq prim(b)	-
Ban I	6	Dra III	-	Mlu I	-	Sec I	28
Ban II	7	Drd I	-	Mnl I	107	SfaN I	29
Bbe I	2	Dsa I	4	Msc I	3	Sfc I	9
Bbs I	12	Eae I	11	Mse I	12	Sfi I	1
Bbv I	21	Eag I	4	Msp I	36	Sma I	2
Bbv II	12	Eam1105 I	3	Mun I	4	SnaB I	1
Bcl I	4	Ear I	8	Nae I	3	Spe I	-
Bcn I	17	Ecl136 II	2	Nar I	2	Sph I	1
Bgl I	5	Eco47 III	4	Nci I	17	Spl I	-
Bgl II	3	Eco57 I	7	Nco I	1	Ssp I	3
Bpm I	5	EcoN I	1	Nde I	1	Stu I	1
Bpu1102 I	6	Eco0109 I	4	Nhe I	2	Sty I	7
Bsa I	5	EcoR I	-	Nla III	29	Taq I	40
Bsa I	6	EcoR II	20	Nla IV	22	Tfi I	21
BsaB I	2	EcoR V	4	Not I	-	Tth111 I	-
BsaH I	7	Esp I	6	Nru I	1	Tth111 II	9
BsaJ I	28	Esp3 I	5	Nsi I	3	Xba I	2
Bsg I	7	Fnu4H I	40	Nsp7524 I	9	Xca I	1
BsiE I	5	FnuD II	30	NspB II	10	Xcm I	1
BsiW I	-	Fok I	26	NspH I	9	Xho I	3
Bsl I	31	Fsp I	2	Pac I	-	Xma I	2
Bsm I	5	Fsp II	2	PaeR7 I	3	Xmn I	6
BsmA I	18						

Enzyme	Site	Use	Site position (Fragment length) Fragment order		
Afl II	c/ttaag	1	1(2404) 2	2405(6597) 1	
BamH I	g/gatcc	1	1(7329) 1	7330(1672) 2	
BspH I	t/catga	1	1(8443) 1	8444(558) 2	
Bst1107 I	gta/tac	1	1(1327) 2	1328(7674) 1	
BstX I	ccannnnnn/ntgg	1	1(8342) 1	8343(659) 2	
EcoN I	cctnn/nnnagg	1	1(4850) 1	4851(4151) 2	
Nco I	c/catgg	1	1(5921) 1	5922(3080) 2	
Nde I	ca/tatg	1	1(8830) 1	8831(171) 2	
Nru I	tcg/cga	1	1(8599) 1	8600(402) 2	
PflM I	ccannnn/ntgg	1	1(3412) 2	3413(5589) 1	
Pme I	gttt/aaac	1	1(8384) 1	8385(617) 2	
Pml I	[illegible]	1	1(6131) 1	6132(2870) 2	
Sal I	g/tcgac	1	1(1540) 2	1541(7461) 1	
Sfi I	ggccnnnn/nggcc	1	1(570) 2	571(8431) 1	
SnaB I	tac/gta	1	1(335) 2	336(8666) 1	
Spl I	gcatg/c	1	1(6660) 1	6661(2341) 2	
Stu I	agg/cct	1	1(4497) 2	4498(4504) 1	
Xca I	gta/tac	1	1(1327) 2	1328(7674) 1	
Xcm I	ccannnnnn/nnnntgg	1	1(7707) 1	7708(1294) 2	
Age I	a/ccggt	2	1(4021) 2	4022(4417) 1	8439(563) 3
ApaL I	g/tgcac	2	1(5423) 1	5424(1621) 3	7045(1957) 2
Bbe I	ggcgc/c	2	1(2239) 3	2240(4097) 1	6337(2665) 2
BsaB I	gatnn/nnatc	2	1(1437) 3	1438(2044) 2	3482(5520) 1
BspE I	t/ccgga	2	1(1936) 2	1937(6864) 1	8801(201) 3
BspM II	t/ccgga	2	1(1936) 2	1937(6864) 1	8801(201) 3
BstB I	tt/cgaa	2	1(1762) 3	1763(5037) 1	6800(2202) 2
BstE II	g/gtnacc	2	1(4017) 1	4018(1021) 3	5039(3963) 2
Bsu36 I	cc/tnagg	2	1(1001) 3	1002(2744) 2	3746(5256) 1
Dra I	ttt/aaa	2	1(6428) 1	6429(1957) 2	8386(616) 3
Ecl136 II	gag/ctc	2	1(3326) 2	3327(3418) 1	6745(2257) 3
Fsp I	tgc/gca	2	1(3844) 1	3845(2986) 2	6831(2171) 3
Fsp II	tt/cgaa	2	1(1762) 3	1763(5037) 1	6800(2202) 2
Gsu I	ctggag	2	1(5160) 1	5161(3698) 2	8859(143) 3
Hpa I	gtt/aac	2	1(4660) 1	4661(2195) 2	6856(2146) 3

Strategy for 3-way fusion PCR



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For example, here is how two of the primers will be oriented:

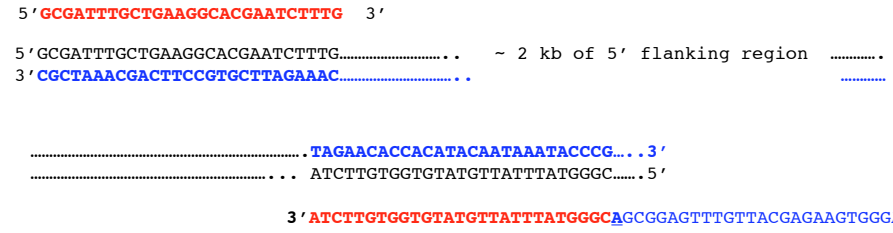
5' FOR: 5'GCGATTTGCTGAAGGCACGAATCTTTG 3'

+

5' REV + pyrG A.f. marker tail:

5'GAGGGTGAAGAGCATTGTTTGAGGCGACGGGTATTTATTGTATGTGGTGTTCTA 3'

5' Forward primer



5' Reverse + marker tail

…by the end of Week 2, students have learned how to design PCR primers for 3-way fusion PCR…

Table 2: Primers used in PCR for the amplification of both 5' and 3' AN3620.2 flanking regions. The underlined and color coded portions represents the part of the sequence overlapping with *pyrG A.f.*

Name	Primer Sequence	Tm °C	Annealing t °C
5'FOR	5'GCGATTTGCTGAAGGCACGAATCTTTG 3'	72.8	62.0
5'REV	5'GAGGGTGAAGAGCATTGTTTGAGGCGACGGGTA TTTATTGTATGTGGTGTTCTA 3'	86.9	62.0
3'FOR	5'CATCACGCATCAGTGCCTCCTCTCAGACAGCAGT AAGGGATGATTTGGAGTGAA 3'	90.7	62.0
3'REV	5'ACTGCCTATGATACTTGGAAGCGTCTCA 3'	68.0	62.0

The sequences in **black** are gene-specific and will differ for each deletion

Two of the primers will incorporate the *pyrG* A.f. sequences shown in blue

Biology 351 Laboratory
Week 3

Tuesday, 9/12/06

Part I of 3-way fusion PCR:

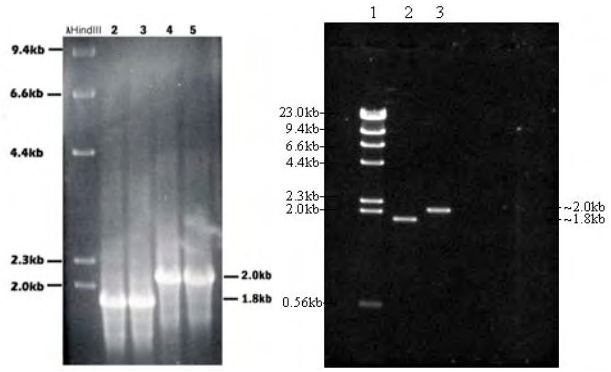
Amplify *A. nidulans* 5' and 3' flanking regions

Learning goals for this week's lab:

1. **Prepare four oligonucleotides for PCR –** dilute to appropriate concentrations.
2. **Plan two PCR reactions –** design two PCR reactions and understand the role of each component in the reactions.
3. **Learn how to program and use the Bio-Rad thermal cyclers for PCR**
4. **Perform the first set of amplifications to obtain 5' and 3' flanking genomic DNAs**
5. **Cast a large-format gel, electrophorese the amplified DNAs, and excise the desired PCR products from the gel.**
6. **Use the QIAEX II kit to extract and purify each 5' and 3' flanking PCR product.**
7. **Check for recovery of your PCR products using an agarose mini-gel.**
8. **Quantify recovery of your PCR products using the DyNAQuant 200 Fluorometer.**
9. **Use Powerpoint to assemble a publication-quality figure, including a complete legend, for your DNA gels; and perform a simple *in silico* analysis of your favorite gene.**

Week 3: PCR Step I - Amplify & purify flanking DNA regions on either side of the target gene

Figure 2A: PCR amplification of *rad1* 3' and 5 flanking regions: *Aspergillus nidulans* strains used were PCS439 (*riboA*1 *yA*2) and SWJ400 (*pabaA*1 *yA*2; *nimO*18). ~2.01kb and ~1.83kb *rad1* 3' and 5' flanking regions were amplified in two separate PCR using 2 sets of primers 3'FOR and 3'REV, 5'FOR and 5'REV. Primers 5'REV and 3'FOR incorporated complementary *pyrG* tails, which were to be used in a 3-way fusion PCR with *pyrG*. **Key to lanes: 1** λ*Hind*III markers (625ng) , **2,3 *rad1*** 5' flanking region, **4,5 *rad1*** 3' flanking region **Figure 2B:** 3' and 5' *rad1* flanking PCR products were excised and QIAEX purified. Duplicate products were pooled and quantity was assessed by electrophoreses of 2μof each amplified flanking region. **Key to lanes: 1** λ*Hind*III markers, **2** Pooled ***rad1*** 5' flanking DNA, **3** Pooler ***rad1*** 3' flanking DNA.



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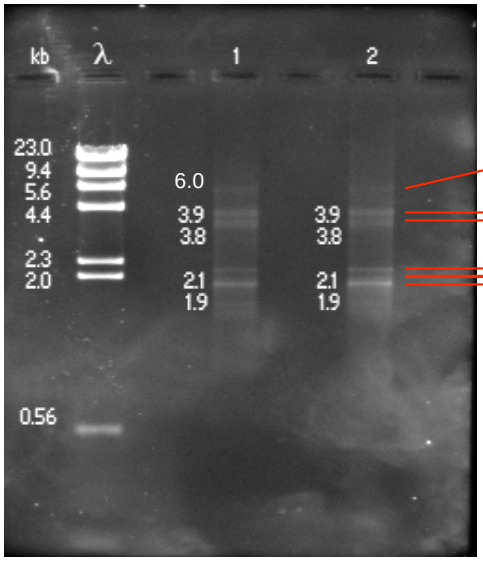
Week 4: PCR Step II - 3-way fusion PCR

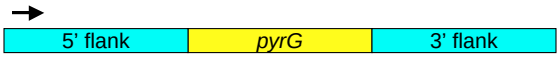


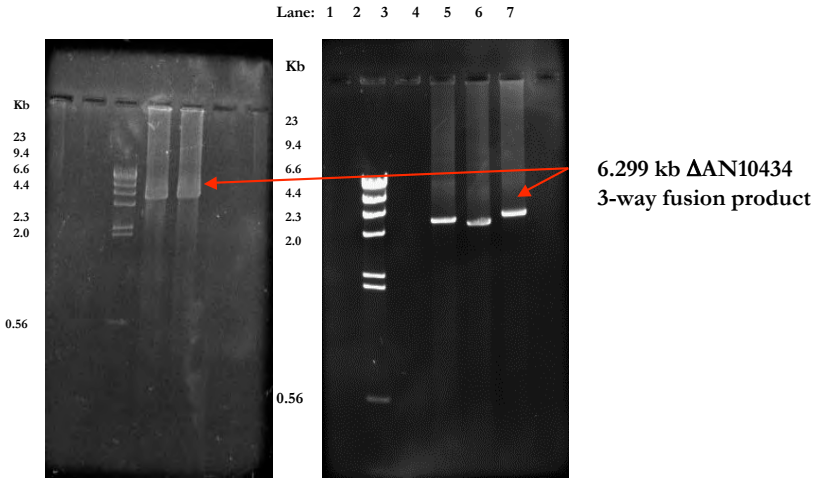
6.0 kb 3-way fusion

2-way fusion products

Individual flank DNAs + *pyrG* A.f.

Week 5: PCR Step III - Nested PCR to prepare for transformation





6.299 kb ΔAN10434 3-way fusion product

Week 6: Transform *A. nidulans* with deletion constructs

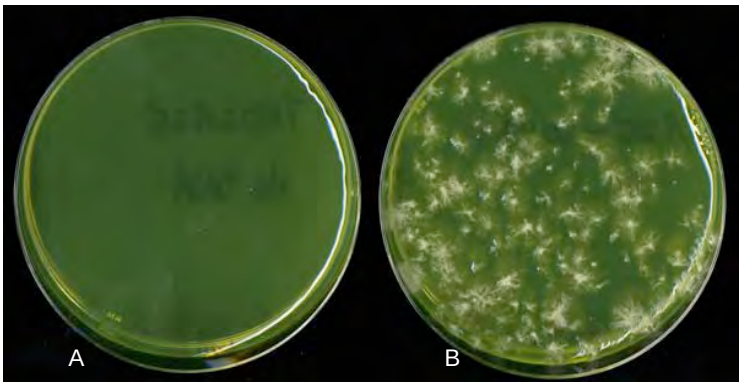
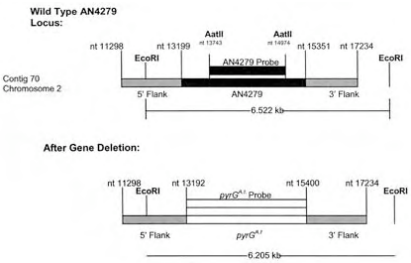


Figure 5. Successful transformation of *A. nidulans* with ΔAN10434 deletion construct

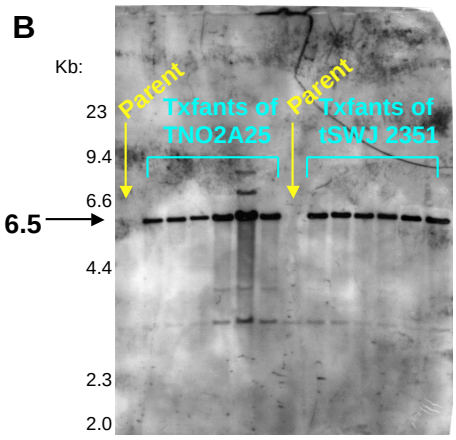
The purified 3-way fusion construct was transformed into TN02A25 (pyrG89 pabaA1; ΔnkuA::argB; riboB2). Transformants were plated on medium lacking uridine and uracil as a means to screen for the successful integration of the *pyrG A.f.* fusion construct. Equal numbers of protoplasts were plated in A and B. Plates were grown for 3 days at 29ºC. (A) No DNA control transformation. (B) Transfomation with ΔAN10434.

Weeks 7-11: Purify transformants, prepare gDNAs, identify deletions by Southern blotting

A



B



*EcoR*I digests probed with *pyrG A.f.*

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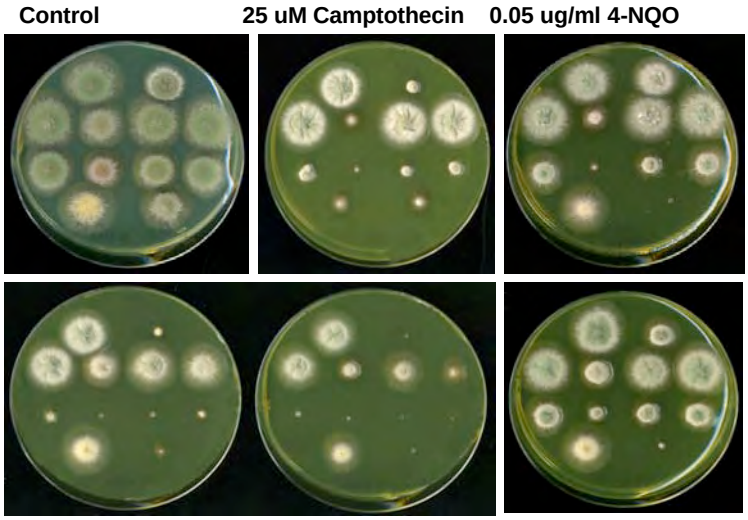
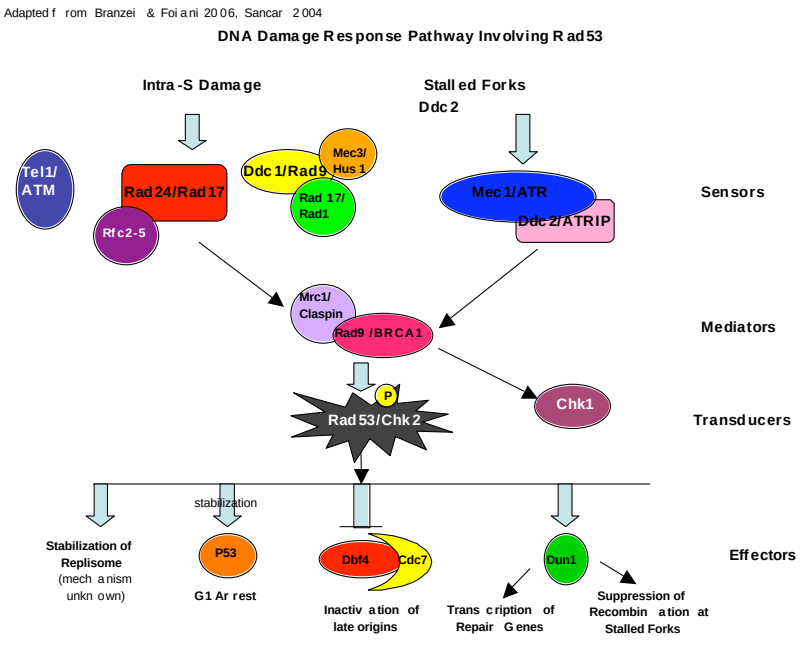
Week 12: Phenotypic analysis to test for sensitivity to DNA damage agents





Summary:

To complete the project, students…

1. **Create a pathway diagram to describe the function of their gene within the context of DNA damage control**

2. **Write a 1-2 page abstract that describes everything known about their gene**

3. **Write a complete scientific paper, as-for-publication, to document their semester-long efforts**

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MOLECULAR GENETICS (Biology 351) - Fall, 2006

Course objectives: The Central Dogma of Molecular Biology describes how information flow occurs, from DNA to RNA to protein, in all living systems. This course examines the mechanisms by which the elaborate processes of life, governed by complex machines made of protein and/or RNA, are derived from a linear, digital DNA codex; how the integrity of the genetic endowment is maintained and how DNA is replicated faithfully despite constant injury and the threat of mutation; how sophisticated regulatory mechanisms are able to sense changing environment and respond by altering the genetic program; and how disease states may perturb both the overall structure of the genetic material and the control of individual genes. By examining current research in topical areas of molecular biology, and by applying methods of molecular biology to study eukaryotic gene function, students will deepen their understanding and appreciation of the beauty, complexity, and subtlety of life at the level of molecule and gene.

Student learning objectives: Students in this course will develop the following competencies:

1. Develop a complete and detailed understanding of the Central Dogma of Molecular Biology, elaborated through the study of current research problems; understand the mechanisms by which DNA is replicated, transcribed, translated, repaired, and recombined; understand the basic techniques for studying nucleic acids and proteins, and how they are applied.

2. Develop an appreciation for the complexity of living processes at the molecular level; understand how gene expression and protein function can be rapidly altered in response to environmental stimuli, and how these changes, controlled by intricate regulatory circuits, modify behavior at the level of cell and organism.

3. Become proficient at reading, analyzing, and understanding original research articles in the field of molecular biology; develop an understanding and appreciation for the *experimental* approach, *i.e*., how the varied tools and methods of molecular biology can be used to ask, and answer, scientific questions that reveal new insights about biological processes.

4. Become proficient at laboratory techniques for DNA isolation, manipulation, cloning, and analysis.

5. Develop the ability to write a complete scientific paper, as for publication. During the writing process, students will learn how to (a) synthesize the data from a variety of experiments into a cohesive summary, (b) analyze their experimental results, and (c) integrate their findings into a theoretical framework, namely checkpoint control and the DNA Damage Response (DDR) in eukaryotes.

Capstone experience: *Molecular Genetics* integrates learning from a number of different foundational courses, including *Genetics* (Bio 211), *Cell Biology* (Bio 212), *Bioinformatics* (Bio 251), *Microbiology* (Bio 230), and *Biochemistry* (Chem 333, Bio/Chem 334). In addition, *Molecular Genetics* shares significant disciplinary approaches and principles with *Immunobiology* (Bio 332) and *Evolution* (Bio 314). Furthermore, *Molecular Genetics* provides the opportunity for students to demonstrate proficiency at communication conventions of their major, through the writing of a comprehensive scientific paper. This comprehensive paper is linked with the semester-long research project that forms the basis for the laboratory component of the course. For these reasons, *Molecular Genetics* may be used in fulfillment of the **capstone experience** for majors in Biology or Biochemistry and Molecular Biology.

MOLECULAR GENETICS (Biology 351) - Fall, 2006

Lecture:	MWF 9:00 am	356 Science Center (Chemistry seminar room)
Laboratory:	T 1:10 - 5 pm	252 Science Center
Instructor:	Steve James	255 Science Center x6170 e-mail: sjames@gettysburg.edu

Text: Molecular Biology, 3rd edition. By Robert F. Weaver. McGraw-Hill, 2005

Additional readings will be assigned, copies of which will be housed in 252 SC.

Lectures: Advance preparation and class participation is expected. Textbook and reserve readings must be completed prior to the class for which they are assigned. Problem sets will be assigned during the semester to aid in learning and exam preparation. These problem sets will be graded.

Laboratory: The laboratory consists of a multi-faceted, semester-long project in which the student will use molecular genetic techniques to perform a gene deletion and then characterize the phenotypic consequences of the gene knockout. Due to the length and scope of some experiments, students will work semi-independently and will occasionally need to work outside of the scheduled laboratory. The student will write a comprehensive scientific paper to analyze the results of the project, and integrate these results into a theoretical framework related to the maintenance of genome integrity.

Course Grade:

Three one hour exams	13.3% each
Final exam	15% (1/4 course review)
Homework and quizzes	10%
Lab assignments	20%
Laboratory paper	15%

(Note: overdue assignments will reduce by one letter grade for each day late)

Attendance in lecture and lab is mandatory. A student with more than three unexcused absence from lecture, or from one laboratory, will be invited to leave the course.

BIOLOGY 351 – FALL, 2006

Molecular Genetics

COURSE SCHEDULE

Steve James
255 Science Center
337-6170
sjames@gettysburg.edu

Science Center 356
MWF 9 – 9:50

LAB: 252 SC, T 1 - 5

Date	Lecture topic	Laboratory
Aug 28 30 Sept 1	First class: Orientation to Laboratory project How (and why) to delete a gene (continued) CDMB: Alkaptonuria unites molecular biology from fungi to man	Choose partner: Begin designing gene deletion - bioinformatic surveys
Sept 4 6 8	Molecular and *in silico analysis*: homogentisate dioxygenase gene Building blocks: discovery of nucleic acid structure Nucleic acid chemistry	Design and order PCR primers for gene deletion (3-way PCR)
Sept 11 13 15	Nucleic acid metabolism and related disorders Physical behavior and topology of DNA molecules Topoisomerases, DNA topology, cancer & bioterrorism	Polymerase Chain Reaction (PCR), Step 1
Sept 18 20 22	Analysis of Chen *et al*. 1996. Gyrases, antibiotics, & DNA cleavage Analysis of Chen *et al*. 1996. Gyrases, antibiotics, & DNA cleavage Translation of proteins in prokaryotes & eukaryotes: initiation	Step 2 of 3-way fusion PCR to prepare gene deletion construct
Sept 25 27 29	**EXAM 1** Translation of proteins: elongation and termination VIDEO: Human genome project	Final step of fusion PCR/clone construct - DNA-DNA ligation: create recomb. DNA
Oct 2 4 6	Ribosomes and transfer RNA Genetic Code: degeneracy, wobble, & tRNA suppression The other Genetic Code: Aminoacyl tRNA synthetases & evolution	Restriction enzyme analysis of recombinant plasmids
Oct 9 11 13	**READING DAY** Post-translational controls: MPF and the cell cycle Analysis of Gould & Nurse, 1989: Phosphorylation/dephosphorylat'n Control of cell cycle progression by regulated proteolysis	DNA-mediated trans-formation of *Aspergillus* - *purify transformants*
Oct 16 18 20	Analysis of papers: the Anaphase Promoting Complex (APC/C) Checkpoint control and the cell cycle Checkpoint control: the DNA Damage Response (DDR)	Prepare genomic DNA from transformants; screen phenotypes
Oct 23 25 27	**EXAM 2** The replicon and initiation of DNA replication in *E. coli* *dnaA*, *dnaB*, *dnaC* and the initiation of DNA replication Control of	Southern blot analysis of transformant DNA Part I

	30	The replisome and DNA synthesis in *E. coli*	Southern blot analysis of transformant DNA Part II
Nov	1	DNA synthesis in eukaryotes: G1/S transition	
	3	The CHIP assay: Analysis of Aparicio, Weinstein, and Bell (1997)	
Nov	6	Analysis of Aparicio, Weinstein, and Bell (continued)	More phenotypic screening; prepare figures for final paper
	8	Maintaining genome integrity: mutation and DNA repair	
	10	Base excision repair and Nucleotide Excision Repair	

Nov	13	Mismatch repair in prokaryotes versus eukaryotes	Finish experiments; draft final laboratory paper
	15	Recombination repair and the SOS response	
	17	Double-strand break repair	
Nov	20	**EXAM 3**	Submit draft of paper
	22	**THANKSGIVING RECESS**	
	24	**THANKSGIVING RECESS**	
Nov	27	Prokaryotic transcription: RNA polymerase & promoters	Field trip: Armed Forces DNA Identif'n Laboratory (AFDIL)
	29	Gene Expression in prokaryotes: σ factors and regulators	
Dec	1	The *lac* and *trp* operon models for gene regulation	
Dec	4	*lac* operon: DNA-protein interactions	Finish laboratory paper
	6	*lac* operon (continued)	
	8	Terminating transcription: Attenuation in the *trp* operon	
FRI, DEC	**15**	**FINAL EXAM, 1:30 - 4:30 pm**	

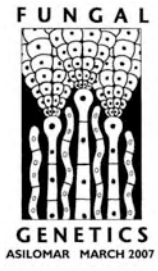
Additional topics that could be covered in place of other syllabus topics:

The end-replication problem: Telomere structure/function
Telomeres and telomerase in cancer and aging
Telomeres and telomerase: analysis of research papers

MILLSAPS COLLEGE

Teaching with Fungi: From College Freshmen to Seniors

Sarah Lea McGuire



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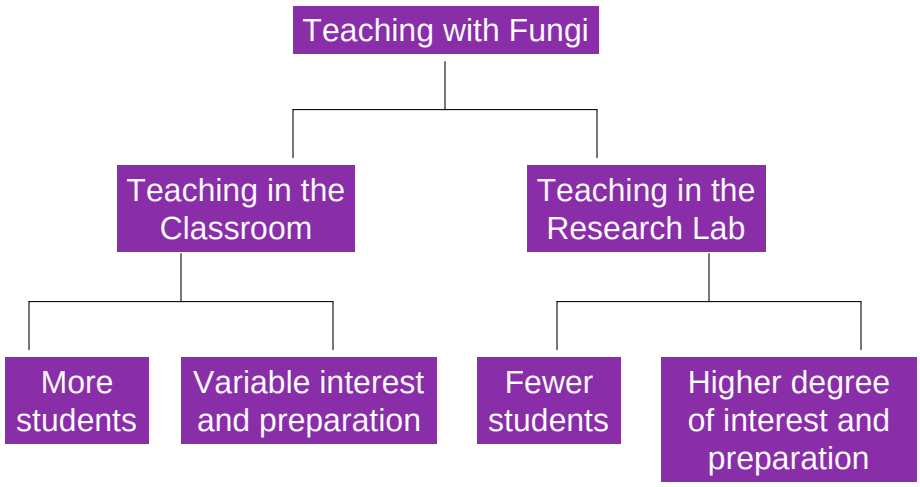

MILLSAPS COLLEGE

Teaching Goals

- Teaching in the Classroom
  - Demonstrate cellular processes, structures, phenomena; teach scientific method and reasoning
  - Stimulate Imagination
- Teaching in the Research Lab
  - Stimulate Imagination
  - Teach research methods, data analysis, experience scientific community

MILLSAPS COLLEGE

Teaching in the Classroom

Freshmen

Sophomores

Juniors/Seniors

http://www.fgsc.net/teaching/labfungi.htm

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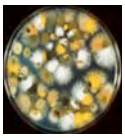
MILLSAPS COLLEGE

Types of Activities

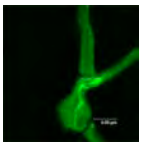
Freshmen

- General aseptic technique

- Scientific method/classic experiments
- Genetics: Analysis of cross progeny



- Cell Biology: cytoskeleton, nuclei, and other subcellular structures



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Lessons Learned

Teaching in the Classroom: What Doesn't Work

Freshmen

- Using unclear phenotypes
- Asking students to design/conduct a series of experiments with several new skills
- Expecting students to understand how to make solutions before you teach them
- Asking students to write a full scientific paper on their experiments before they've learned the various parts of a paper

Teaching in the Classroom: What Works

- Choose easily visualized markers
- Teach students one skill at a time
- Prepare solutions/media for the students
- Have students analyze results of experiments
- Teach writing one section at a time

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Types of Activities

Sophomores

- **Biochemistry: Classic location of point of block of a biosynthetic pathway (*Neurospora*)**
- **Genetics: Gene mapping, epistasis, mutant analysis (*Aspergillus*)**
- **Molecular Biology: Transformation (*Saccharomyces*)**
- **Cell Biology: Effects of mutations on cell structures (*Aspergillus, Saccharomyces*)**

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Lessons Learned

Teaching in the Classroom: What Doesn't Work

Sophomores

- **Asking students to design a complete set of experiments at the beginning of the semester**
- **Having students prepare all of their media**
- **Using unclear phenotypes**

Teaching in the Classroom: What Works

- **Have students carry out and analyze all experiments**
- **Link weekly activities to teach students how to design a progression of experiments aimed at a specific goal**
- **Teach literature review and write a complete paper in sections**

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Types of Activities

Juniors/Seniors

- **Combine molecular, genetic, biochemical, and cell biology techniques through semester-long projects**
- **Generate/analyze sets of double mutants and to determine/characterize genetic interactions (*Saccharomyces*)**
- **Use strains from the research laboratory and have students design/conduct experiments to analyze these**

MILLSAPS COLLEGE

Lessons Learned

Teaching in the Classroom: What Doesn't Work

Juniors/Seniors

- **Requiring too *little* of the students**
- **Unclear expectations**
- **Too little support**

Teaching in the Classroom: What Works

- **Set high expectations**
- **Approve experimental design before allowing students to perform experiments**
- **Have students prepare scientific papers reporting their findings**
- **Have students make oral or poster presentations of their work**

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Teaching in the Research Lab

Goals

Teach critical thinking skills and scientific thought

Help students master a variety of techniques and understand their applications

Help students explore scientific research as a career prospect

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Teaching in the Research Lab

Teaching in the Research Lab: What Works

- **Provide students with projects that have attainable goals and have a high likelihood for success**
- **Involve students in all aspects of the lab—from media prep to literature review and experimental design**
- **Start less experienced students with appropriate activities**
- **Make certain that students understand how their project fits into the overall goals of the lab**
- **Provide students with opportunities to present their data at scientific meetings**

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MILLSAPS COLLEGE

Millsaps College Fungal Genetics Research Team

Research for now…

Research for the future.

Yulon Stewart | Michael Yablick | Sarah Lea McGuire | John Gibson | Kirk Jackson

Bringing student inquiry and research into your courses by collaborating with graduate research consultants or advanced undergraduates

Patricia Pukkila
Professor of Biology and
Director, Office of Undergraduate Research

XXIV Fungal Genetics Conference, 2007

Goals for session

- Assumptions
- Use collaborative learning model to address a complex problem
- Resources

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Assumptions

- Goals as educators include facilitating student progression from “novice” to “expert”
- We can each recall an example from our own experience when we made such a transition

Start with your experience

- Grads and postdocs: when you first made the transition to viewing yourself as a professional
- Insight: “they can’t be right about that”
- Insight: “whoops, there is a better way for me to think about this”

2 minutes to discuss your novice→ expert transition with person next to you

Problem for today

Students rely on memorization, assimilation, imitation ("novice" approaches)

What might work to encourage original inquiry ("expert" approaches)?

5 minutes to discuss with person next to you

Summary

- Motivation to actually learn the material and be excited to learn more
- Guide the students to design the experiment but to be flexible when things don't work and be willing to learn from that and know that is ok and a part of the process. Don't be afraid to try! Experiment works, may learn something new.
- Could give experiment that won't work to learn and trouble-shoot (boost ability)

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Summary - 2

- Give project where chance to revise and redo to improve and make better and build that into the process. Allow them to help and make the process better.
- Avoid detachment from the real world. Make work applicable and tied to the real world.
- Students having the ability to present or teach their work and to demonstrate their knowledge to others. The realization that they know what they are talking about.

Resources-GRC model

- Collaborative teaching enables collaborative learning (value of peer perspectives)
- Graduate research consultants (GRCs) enable separation of educator/evaluator roles (GRCs do not participate in grading)
- GRC model encourages small-scale changes (student presentations→student proposals)

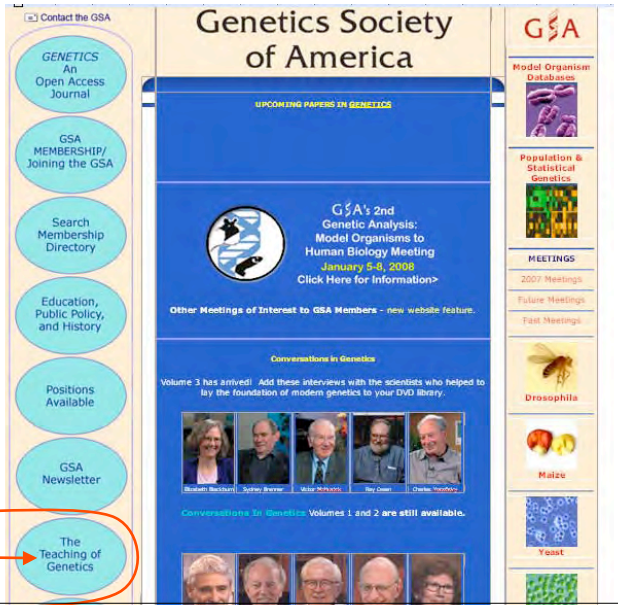
Resources-Campus reaccreditations

THE UNIVERSITY
OF NORTH CAROLINA
AT CHAPEL HILL

MAKING CRITICAL CONNECTIONS

QUALITY ENHANCEMENT PLAN

Resources: publications http://www.genetics-gsa.org/



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Dynamics I experienced

- Joys of moving from novice to expert in science
- Peer discussion (importance of trust)
- Collaborative learning
  - Focus on the different ways we can seek solutions
  - Develop our abilities to improve as educators

Thank you!