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Abstract

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GENOME STRUCTURE AND MAINTENANCE

COMPARATIVE AND FUNCTIONAL GENOMICS

Genome reconstruction and gene expression for the rice blast fungus, *Magnaporthe grisea.*
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Rice blast disease, caused by *Magnaporthe grisea*, is one of the most devastating threats to food security worldwide. The fungus is amenable to classical and molecular genetic manipulation and is a compelling experimental system for elucidating numerous aspects of pathogenesis, including infection-related morphogenesis, host species and cultivar specificity, and signaling pathways. In 1998, an international consortium (IRBGP) was established to sequence the rice blast genome. For this initiative, we used a 25X large insert (130 kb) *HindIII* BAC library to construct a physical map of the genome. BAC clones were fingerprinted and assembled into 188 contigs. These were aligned into a physical map by anchoring to mapped RFLP markers. Chromosome 7 (4.2 Mb) has been studied in the greatest detail and a set of 42 BAC clones representing a minimum tiling path covering >95% of the chromosome has been deduced. The sequence of one BAC clone (6J18) has been completed. The entire BAC library was end sequenced providing

sequence tag connectors (STC) every 3-4 kb across the genome. A federated database integrating physical, genetic and expression data from relational and object-oriented databases is being developed. We have initiated a draft sequence (~5X coverage) of chromosome 7 using the "BAC by BAC" approach coupled with information from our STC/fingerprint databases. A comprehensive EST program has been launched. 30,000 ESTs will be derived from 8 cDNA libraries prepared from different stages of growth and development as well as cells subjected to various stress conditions. A set of ~5,000 ESTs representing unique genes will be further sequenced. The current status of the genome project and database development will be presented.

Partial reconstruction of the metabolic capacity of *Pneumocystis carinii* through genomics and identification of potential therapeutic targets. Melanie T. Cushion[1], Bradley E. Slaven[1], Jonathan Arnold[2], John Wunderlich[2], Chuck Staben[3], Tom Sesterhenn[1] and A. George Smulian[1].

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Pneumonia caused by *Pneumocystis carinii* (**PcP**) remains the leading opportunistic infection associated with AIDS patients, even in the era of Highly Active Anti-Retroviral Therapy (HAART). Despite concerted efforts to identify additional therapeutic agents, trimethoprim-sulfamethoxazole (TMP-SMX) continues to be the standard prophylactic and therapeutic modality in use today, as it was over 20 years ago. Recently, treatment failures of TMP-SMX were linked to mutations in the target genes in *P. carinii* making the search for new targets a priority. Unlike any extant fungus, *P. carinii* possesses a single copy of the nuclear ribosomal RNA locus and has little to no ergosterol. It is refractory to standard anti-fungals, including amphotericin B and standard azoles such as fluconazole. These data suggest there are distinct differences in some metabolic pathways of other fungi and Pc.

Genetic data resulting from the *Pneumocystis* Genome Project offers promise for development of new drug targets. Analysis of sequences from a partial Expressed Sequence Tag (EST)-, cosmid end sequence-, and cosmid library databases by homology searches revealed the presence of several metabolic pathways with drug targeting potential, including sterol and heme biosynthesis. *P. carinii* appears to possesses most of the genes in the sterol biosynthetic pathway, including *ERG4,* the final enzyme that converts ergosta-5, 7, 22, 24, (28)-tetraen-3, B-ol to ergosterol, and squalene synthase (*erg9*), squalene epoxidase (*ERG1*), lanosterol cyclase (*ERG7*) and SAM:SMT (*ERG6*). Assessment of the efficacy of compounds targeting the enzymatic steps along the sterol biosynthetic pathway using a short term drug screening assay based on measurement of ATP by bioluminescence correlated with the presence or absence of the gene in the sequence databases. These data will be used to formulate combination therapies that will be assessed in pre-clinical trials using rodent models of infection.

Phytophthora genomics. Brett M Tyler[1], Felipe R. Arredondo[1], Howard S. Judelson[2], Ralph A. Dean[3], Peter Hraber[4], Mark E.Waugh[4], Bruno W. Sobral[5], Callum J. Bell[4], Dinah Qutob[6] , Mark Gijzen[6]. [1]Department of Plant Pathology, University of California, Davis, CA95616; [2]Department of Plant Pathology, University of California, Riverside CA 92521; [3]Fungal

Genomics Laboratory, NC State University, Raleigh, NC 27695-7251; [4]National Center for Genome Resources, Santa Fe, New Mexico 87505; [5]Virginia Bioinformatics Institute, Virginia Tech University, Blacksburg, VA24061; [6]Agriculture Canada, London Research Centre, London, Ontario N5V 4T3, Canada.

The more than 40 species of the oomycete *Phytophthora* cause serious diseases of a huge range of crop and ornamental plants. To facilitate isolation of infection-related genes from the soybean pathogen *Phytophthora sojae*, we are constructing a BAC contig of the entire genome, using a hybridization fingerprinting strategy with random probes, most of them repetitive, and with ESTs. Computer software has been developed to collect and analyze the data. At present, 19% of the BACs have received the minimum number of hybridization hits needed to establish contigs. Of these BACs, 34% have been placed into contigs. We have confirmed the authenticity of three of the largest contigs by HindIII digestion. With the long term goal of sequencing the entire genome of *P. sojae* and selected sequences from other *Phytophthora* species, such as *P. infestans* we have established the *Phytophthora* genome initiative (PGI). We have begun preliminary sequencing of a 200 kb BAC contig spanning two avirulence genes from *P. sojae*. Sequencing of the first 60 kb BAC reveals that the gene density is extremely high. Both the sequencing data and the BAC hybridization data suggest that the *P. sojae* genome is composed of gene-rich regions separated by regions rich in repetitive sequences. Sequencing of 55,000 new ESTS from *P. sojae* and *P. infestans*, and development of a synteny map of the two species, has recently begun, funded by USDA-IFAFS.

Chromosomes II and V of *Neurospora crassa.* Ulrich Schulte, Institute of Biochemistry, Heinrich-Heine-University Dusseldorf, Germany

As part of the German *Neurospora* Genome Project the chromosomes (LG) II and V with a total of 14 Mbp were sequenced. Cooperating partners in the project are Jöörg Hoheisel (DKFZ, Heidelberg), Gerald Nyakatura (MWG Biotech, Ebersberg) and H. Werner Mewes (MIPS, Martinsried). The web accessible MIPS *Neurospora crassa* data base (MNCDB) provides the assembled sequences as well as the identified and annotated open reading frames. More than 3000 genes have been identified sofar. At least 13.000 genes are expected for the entire genome. The deduced proteins are classified according to matches in sequence data bases and attributed to functional categories based on the identified relatives. Concurrently the protein sequences are analysed using the PEDANT system employing a wide spectrum of sequence analysis and structure prediction tools such as attribution of proteins to protein-superfamilies, secondary structure, transmembrane regions, coiled-coil regions etc. Sequencing and annotation data are provided in a comprehensive and multidimensional way. Tools to explore and query the information have been implemented and will be extend further. Generous funding of the project by the Deutsche Forschungsgemeinschaft is greatfully acknowledged.

http://www.uni-duesseldorf.de/WWW/MathNat/biochem/genome.shtml

http://www.mips.biochem.mpg.de/proj/neurospora/

FUNGAL-HOST INTERACTIONS

The *TOX2* locus of *Cochliobolus carbonum* for cyclic peptide biosynthesis: genomic organization and the basis of auto-resistance. Jonathan D. Walton[1], Dipnath Baidyaroy[1], Gerald Brosch[2], and Stefan Graessle[2] [1]DOE-Plant Research Laboratory, Michigan State University, E. Lansing, MI 48824 [2]Department of Microbiology, University of Innsbruck Medical School, Austria

The high virulence of the filamentous fungal pathogen *C. carbonum* on maize of genotype *hm/hm* is due to production of a cyclic tetrapeptide, HC-toxin (cyclo[D-Pro-L-Ala-D-Ala-L-2-amino-9,10-epoxi-8-oxodecanoic acid]). Cyclic tetrapeptides containing Aeo are also known to be made by five other unrelated filamentous fungi. In crosses, production of HC-toxin is controlled by a single locus, *TOX2*. At the molecular level *TOX2* spans at least 560 kb and contains multiple copies of multiple genes. The central gene of *TOX2*, *HTS1*, contains a 16-kb ORF that encodes a tetrapartite 570-kDa non-ribosomal peptide synthetase. Other characterized genes of *TOX2* include *TOXA*, encoding a putative HC-toxin efflux carrier; *TOXE*, encoding a unique pathway-specific transcription factor, and *TOXG*, encoding an alanine racemase. The site of action of HC-toxin is histone deacetylase (HDAC), a key enzyme linking transcriptional regulation (i.e., repression) and chromatin structure in all eukaryotes. We are investigating the mechanism by which *C. carbonum* protects its HDACs from inhibition by its own toxin. The bulk HDAC activity of strains of *C. carbonum* that make HC-toxin ($Tox2^+$) is resistant to HC-toxin and to other HDAC inhibitors, whereas the HDAC activity of other fungi, including *N. crassa*, *A. nidulans*, and yeast, are sensitive. We have obtained evidence for two mechanisms of self-protection in *C. carbonum*. One is an apparent extrinsic protective factor, present only in $Tox2^+$ isolates, that can protect the toxin-sensitive HDAC activity extracted from toxin non-producing ($Tox2^-$) isolates. A second mechanism is an apparent post-translational modification of the major sensitive HDAC activity (ccRpd3) of *C. carbonum*. Whereas yeast has at least five genes that encode NAD^+-independent HDACs (*RPD3*, *HDA1*, *HOS1*, *HOS2*, and *HOS3*), to date only three have been found in filamentous fungi including *A. nidulans* and *C. carbonum*. *ccRPD3* in *C. carbonum* is apparently an essential gene, but strains mutated in *ccHOS2* and *ccHDA1* are viable. The *cchos2* mutant has altered spore morphology, reduced growth on alternate carbohydrate and polysaccharide carbon sources, reduced expression of genes encoding extracellular cell wall degrading enzymes, and reduced virulence on maize. These results indicate that although HDACs normally function as co-repressors of gene transcription, HDACs also have a role in gene activation.

Basidiomycete pathogens: sex, signaling and morphogenesis. J. Kronstad, N. Lee, K. Wake, D. Laidlaw, M. Moniz de Sa, K. Tangen, B. Steen, K. MacDonald, T. Lian, G. Jiang, M. Marra* and S. Jones*. University of British Columbia, Biotechnology Laboratory, Vancouver, B.C., Canada; *B.C. Genome Sequence Centre, B.C. Cancer Agency, Vancouver, B.C., Canada

Experiments with basidiomycete pathogens such as *Ustilago maydis* and *Ustilago hordei* (the smut fungi), and the human pathogen *Cryptococcus neoformans*, have provided considerable insight into the role of mating and signaling in fungal virulence. In the smut species, the a and b mating-type genes control cell fusion as well as subsequent establishment and maintenance of the filamentous, infectious dikaryon. Our comparison of mating-type regions from *U. maydis* and

U. hordei led to the discovery that the MAT region in *U. hordei* is over 500 kb in size. We have recently constructed a physical map of the genome by BAC clone fingerprinting; this map will serve as a platform for additional comparisons of mating-type regions from the smut fungi and for sequencing the MAT region. In the smut fungi, MAPK and cAMP signaling pathways are interconnected with mating-type regulation and play critical roles in virulence. We have characterized downstream targets of cAMP signaling in *U. maydis* and this work identified the product of the hgl1 gene as a potential regulatory factor that controls dimorphism, pigmentation and sporulation. We also recently identified a gene whose transcription is directly or indirectly regulated by the hgl1 product. This gene plays a role in pigmentation and morphogenesis. Investigation of the cAMP pathway also revealed a role for a ras gene in the transition between budding and filamentous growth. Interestingly, mating and cAMP signaling are also involved in virulence and morphogenesis in *C. neoformans.* To gain a deeper understanding of these aspects of virulence, there is a need for genomic resources for *C. neoformans* (and for the smut pathogens). Therefore, we have constructed BAC fingerprint maps of the JEC21 (serotype D) and H99 (serotype A) strains of *C. neoformans*. These maps will contribute to the efforts of an international consortium to obtain the genomic sequence of these strains of *C. neoformans*. As part of this genomic effort with *C. neoformans,* we have also defined the transcriptome of both strains using serial analysis of gene expression (SAGE).

Signal transduction cascades regulating fungal development and virulence. Joseph Heitman, Department of Genetics, Duke University Medical Center

Our studies address how cells sense and respond to their environments. We focus on the opportunistic human fungal pathogen *Cryptococcus neoformans*. This organism is a basidiomycete with a defined sexual cycle involving mating between haploid MAT and MATa cells. Interestingly, the MAT mating type has been linked to prevalence, virulence, and ability to undergo monokaryotic fruiting. Our studies have identified components of conserved signaling pathways that regulate differentiation and virulence. These pathways include a G-protein-cAMP pathway that senses nutrients and a pheromone responsive MAP kinase cascade in which several components are encoded by the mating type loci. Related signaling pathways operate during differentiation and mating in the model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* and during infection of both humans and plants by fungi including *Candida albicans* and *Ustilago maydis*. These findings reveal how common pathways have adapted as organisms diverged to exploit different environments, and illustrate the power of comparative studies to uncover conserved and unique facets of signal transduction.

The arbuscular mycorrhizal fungi and their symbiotic association with plants. Maria Harrison. The Noble Foundation, Plant Biology, Ardmore, OK

Arbuscular mycorrhizas are symbiotic associations formed between plant roots and zygomycetes from the order, Glomales. These fungi, commonly termed arbuscular mycorrhizal (AM) fungi, are obligate symbionts and form associations with plant roots in order to obtain carbon from their plant partner. The symbiosis also benefits the plant and in many cases the AM fungi assist the plant with the acquisition of phosphate from the soil. AM fungi exist in the soil as large multi-nucleate, asexual spores, which are capable of germination and limited hyphal growth in the absence of a plant root. Following entry into a root, the fungus rapidly invades the intercellular

spaces of the cortex and also differentiates within the cortical cells to form highly branched hyphae termed arbuscules. Nutrient exchange (phosphate and carbon) between the symbionts is assumed to occur over the arbuscule /cortical cell interface. Once intra-radical hyphae have developed, the fungus also proliferates in the surrounding soil. AM fungal hyphae are coenocytic and the hyphae in the interior of the root form a single continuum with those in the soil. The internal and external parts of this hyphal system however, exist in highly different environments and show differentiation at the metabolic and functional levels. A model legume, *M. truncatula*, and AM fungi, *Glomus versiforme* and *G. intraradices*, have been selected for studies of the molecular mechanisms underlying development and functioning of the AM symbiosis. High affinity phosphate transporters have been cloned from these AM fungi and studies of the regulation of phosphate transporter gene expression are in progress. EST sequencing and arrays are being used to analyze and profile gene expression in these arbuscular mycorrhizas.

Molecular background to the interaction between parasitic fungi and nematodes

Anders Tunlid, Dag Ahrén, Eva Friman, Tomas Johansson, Maja Lindeblad and Johan Åhman. Department of Microbial Ecology, Lund University, Lund, Sweden.

The nematode-trapping fungi comprise a rather large group of soil living fungi that can infect nematodes by forming special morphological structures (traps) like mycelial networks, adhesive knobs or constricting rings. Following the development of the traps, the fungi infect the nematodes through a sequence of events: attachment to the host surface, penetration, followed by invasion and digestion of the host tissue. We are examining the molecular background to the development of traps by insertional mutagenesis of the fungus *Arthrobotrys oligospora.* The trap of this fungus consists of a three-dimensional network of loops of specialized, differentiated cells. Among ca 5000 mutants, we have isolated a mutant in which the trap development stops after two cell divisions, thus it cannot form a loop and a three-dimensional net. These mutants are presently examined in more detail at genetical and cellular levels. To identify genes and metabolic patways that are uniquely expressed in the trap cells, we have started an EST sequencing project in the fungus *Monacrosporium haptotylum* which captures nematodes with the aid of adhesive knobs. In this fungus, functionally intact trap cells can be isolated from the mycelium. Three different cDNA libraries are analyzed, representing transcripts of mycelium, knobs and knobs infecting *C. elegans.* Finally, we are interested in the role of extracellular proteases in the penetration and digestion of infected nematodes. Using a mutational approach, we have analyzed the function of a cuticle degrading serine protease (PII) in *A. oligospora*. *PII* deletion mutants had significantly lower levels of protease activity compared to the wild-type. In bioassays with nematodes, these mutants developed significantly lower number of infection structures (traps) and had a slightly lower ability to capture nematodes compared to the wildtype. However, the mutants did not appear to be affected in the penetration of the cuticle. The above data, and experiments with "over-expressing" mutants, demonstrate that the activity of PII is important for the digestion of the infected nematodes, thus making nutrients available that the fungus can utilize for the development of new infection structures or, alternatively, for vegetative growth.

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CELL BIOLOGY

The cytoskeleton and tip growth. I. Brent Heath, Biology Department, York University, Toronto, Canada.

Continuous tip growth and the initiation of new tips (branches) are the dominant forms of fungal growth and morphogenesis, yet we lack even a basic understanding of the essential features of both processes. The multi-component, highly dynamic and localized features of tip growth contribute to the difficulties of analysis. Nevertheless, specific patterns of F-actin and tip-high gradients of cytoplasmic calcium ions seem to be universal components of both maintenance and initiation of hyphen tip growth. Among the oomycetes, the dynamic organization of actin filaments and the response of tips to actin disruption with latrunculin B indicate that these filaments function to regulate the extensibility of the tips, localize calcium ion channels and localize exocytosis of apical wall vesicles. The formation of filament arrays characteristic of growing tips early in branch formation indicates their function as one of the early initiation intermediates. In contrast, in eufungi, the apical actin patterns are very different from those of the oomycetes. However, previous observations showing the intimate relationship between the Spitzenkörper and tip growth and the enrichment of actin in the Spitzenkörper demonstrate the importance of actin in tip growth of the eufungi too. The changes in actin patterns and the growth response of eufungal hyphae in latrunculin B indicate that among these hyphae the apical actin may be less important in controlling tip extensibility and more related to exocytosis localization. A spectrin-like protein and or cell wall properties may be more important in extensibility regulation. Interestingly, while latrunculin B clearly affects its predicted target, it does not depolymerize all forms of F-actin and slow polarized tip growth can transiently reform in its presence without formation of a detectable Spitzenkörper. Irrespective of the role of apical actin, tip growth, and thus presumably apical actin, appears to be regulated by a tip-high gradient of cytoplasmic calcium ions which can apparently be generated and maintained by both localized channel-mediated influx from exogenous sources and internal recycling mediated by the apical wall vesicles.

Motile and stationary vacuole compartments in filamentous fungi. Anne E. Ashford. University of New South Wales, School Biological Science, Sydney, Nsw, Australia

Motile vacuole systems have two distinct components: spherical vacuoles that are linked to the plasma membrane and relatively fixed in position, and tubules that extend from these and interconnect them. A number of fluorescent probes that accumulate in the vacuole lumen allow changes in vacuole dynamics in living cells to be recorded by video microscopy. Vacuolar accumulation of probes such as carboxyfluorescein is inhibited by probenecid, indicating that the probe is cleaved from the acetate in the cytoplasm and subsequently taken up across the tonoplast via non-specific anion transporters. Evidence that microtubules and not microfilaments are essential in maintenance and motility of tubular vacuole networks will be reviewed. Tubular vacuole networks are reversibly converted into a series of spherical vacuoles by addition of 40 micromolar oryzalin, while latrunculin B at 25 micromolar has no obvious effect on vacuolar tubules but removes immunocytochemcal staining of the actin caps in the hyphal tips. Under

these conditions growth is inhibited while the vacuole system continues to move forward, resulting in an accumulation of tubules in the tip region. This apparent uncoupling of tip growth and vacuole motility is seen with other growth- inhibiting treaments. The effect of drugs known to perturb processes regulated by GTP-binding proteins on tubular vacuole systems indicates that tubule formation by vacuoles is regulated by GTP-binding proteins and also that different types may be involved in the apical and basal regions of the same hyphal tip cell. The responses are consistent with involvement of a dynamin-like protein in regulation of tubule formation.

Bioimaging of the *Aspergillus nidulans* secretory system. Susan J. Assinder[1], Kelly J. Milward[1], Nicholas D. Read[2] and John H. Doonan[3]. [1]School of Biological Sciences, University of Wales, Bangor, Gwynedd, Wales UK. [2] Institute of Cell and Molecular Biology, University of Edinburgh, Scotland UK, [3] Department of Cell Biology, John Innes Centre, Norwich, UK.

Filamentous fungi are exploited commercially for the production of a variety of secreted proteins. Despite the increasing industrial importance of these organisms, our knowledge of the secretory process is limited and the endomembrane system is poorly characterised. We have used green fluorescent protein (GFP) fusion constructs as vital reporters to study the dynamics of protein secretion *in vivo* in *Aspergillus nidulans*. The *A. nidulans* s*od*^{VI}*C* gene encodes a protein homologous to the -COP subunit of the coatomer complex involved in protein secretion in yeast and higher eukaryotes. The gene is essential and strains carrying the temperature-sensitive s*od*^{VI}*C1* mutation are defective at restrictive temperature in both hyphal extension and nuclear division. The wild-type gene has been cloned by complementation of *sod*^{VI}*C1* from a chromosome VI-specific cosmid library. A chimeric protein containing a plant-adapted GFP fused in-frame to the C-terminus of Sod^{VI}C also complemented the temperature-sensitivity of *sod*^{VI}*C1* and was therefore deduced to be functional *in vivo*. This, and other GFP-tagged constructs, have been used visualise components of the *A. nidulans* secretory pathway using confocal microscopy. GFP fused to a plant ER-retention signal targeted to the endoplasmic-reticulum (ER), whereas the sod^{VI}C:GFP fusion protein showed localisation to putative Golgi bodies concentrated at the hyphal tip. The secretory blocker Brefeldin A led to an accumulation of GFP in the ER, and decreased hyphal tip localisation by the sod^{VI}C:GFP fusion. The data support the view that secretion in filamentous fungi occurs by a mechanism similar to that in other eukaryotes, but with proteins destined for secretion being directed to the hyphal tip.

Struture and function of the Woronin body. Gregory Jedd and Nam-Hai Chua. Laboratory of Plant Molecular Biology, The Rockefeller University

Filamentous fungi have adopted a syncytial mode of cellular organization that allows the movement of cytoplasm and subcellular organelles between cells. This type of organization is likely to provide an advantage based on cooperation between cells, but also carries risks: when hyphae lyse the septal pore must be sealed to maintain cellular integrity. This function is executed by the Woronin body; a dense-core vesicle that rapidly occludes the septal pore following cell lysis. To determine both the origin and precise function of the Woronin body we purified it from *Neurospora crassa* and isolated a peroxisome-targeting signal-1 (PTS1) containing protein that we called HEX-1. HEX-1 is localized to the matrix of the Woronin body by immunoelectron microscopy and a GFP-HEX-1 fusion protein is targeted to the yeast peroxisome in a PTS1 and peroxin dependent manner, suggesting that the Woronin body

originates in the peroxisome. In addition, the expression of *hex-1* in yeast results in the formation of intra-peroxisomal Woronin-body-like structures, suggesting a HEX-1 encoded mechanism of Woronin body-core formation. Deletion of *hex-1* in *N. crassa* eliminates Woronin bodies from the cytoplasm and results in hyphae that exhibit a cytoplasmic bleeding phenotype following cell lysis. Together, these results show that HEX-1 is necessary and sufficient for the formation of the Woronin body-core. In addition, we conclude that the Woronin body is required for the maintenance of cellular integrity following cell lysis. We are currently comparing Woronin body structure and composition in several model fungi. Preliminary results suggesting a mechanism of Woronin body formation will be presented.

COT1 kinase - a regulator of hyphal elongation - can we place it on the MAPK? Oded Yarden, Rena Gorovits, Oshrat Propheta, Saar Cohen and Zipora Resheat-Eini. Department of Plant Pathology and Microbiology , Faculty of Agricultural Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel

Neurospora crassa grows by forming spreading colonies. *cot-1* belongs to a class of *N. crassa* colonial temperature-sensitive (*cot*) mutants, and encodes a Ser/Thr protein kinase, which is highly similar to the mammalian myotonic dystrophy kinase (DMPK). Two COT1 isoforms are produced and can be detected in cytoplasmic, nuclear and membrane fractions of the fungal cell. When grown at restrictive temperature, alterations in cell shape, changes in plasma membrane structure and a reduction in the abundance of the lower MW COT1 isoform were found to occur in a *cot-1* background. The lower MW COT1 isoform was also less abundant in two additional unlinked mutants exhibiting defects in polar growth - *cot-3* and *cot-5* (determined to encode translation elongation factor 2 and an alpha 1,3 mannosyltransferase, respectively), when grown at the restrictive temperatures. A reduction in membrane-associated COT1 was detected at elevated growth temperatures. This was accompanied by a significant increase in proton eflux and a concomitant 2.5-fold decrease in intracellular sodium content. As amending the growth medium with inhibitors of ion pumps, sodium or sorbitol partially suppressed the *cot-1* phenotype, we concluded that cellular osmoticum is altered in *cot-1*. The osmoeffect of sodium and sorbitol and the 3-fold levels of glycerol measured in *cot-1* indicated a possible linkage between COT1 and OS2 (a component of the hyperosmotic stress response MAP kinase pathway). However, as a functional OS2 is not essential for conferring the *cot-1* phenotype and glycerol accumulation (as determined by analysis of a *cot-1*;*os-2* mutant), we suggest that an alternative cascade may be involved.

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GENOME STRUCTURE AND MAINTENANCE

Fungal chromosome rearrangements in the new era. David D. Perkins. Department of Biological Sciences, Stanford University, Stanford, CA

Rearrangements have long served as basic tools for the genetics of eukaryotes, providing information on genome organization and on the behavior of chromosomes in meiosis. They have been used to determine gene sequence and to relate genetic and physical maps, to reveal position

effects and transvection, to examine dosage effects and gene-silencing in segmental aneuploids, to determine relationships between species, and to construct evolutionary trees. Animals and plants have led the way in these studies. The fungi were late starters in the study of rearrangements. Advantageous features of fungi such as haploidy and the survival of all four meiotic products have compensated for the disadvantage of small chromosome size. Knowledge of fungal rearrangements is still fragmentary, however, and their potentialities as experimental tools and as tags in tracing phylogenies are still largely untapped. -- Far from making the use of chromosome rearrangements obsolete, new developments in molecular genetics and genomics can be expected to increase their relevance and usefulness. DNA sequencing is certain to reveal rearrangements that are invisible to classical cytogenetics, as was shown dramatically when the completely sequenced genomes of Saccharomyces and Candida were compared. Existing examples from fungi and other organisms illustrate what may be expected from future research with fungal rearrangements. Rearrangements are already being used in new ways, such as for studying the RIP machinery, intracellular trafficking in heterokaryons, and the restoration of fertility to barren duplications. Rearrangemetns are potentially useful for determining whether segmental domains exist that are subject to epigenetic control. Junction-sequences will provide information on the origin of rearrangements, showing whether recombination occurs between relic transposable elements. Species comparisons will enable conserved, displaced segments to be identified in the genomes of related taxa, while ancient rearrangements can provide unique markers for establishing phylogenetic relationships among distant taxa..

Break-dancing chromosomes: meiosis and DNA repair in Coprinus. Mimi Zolan, Sonia Acharya, Martina Celerin, Jason Cummings, Erin Gerecke, Alex Many, Daniel Maillet, Sandra Merino, Elizabeth Sierra, and Kevin Young Department of Biology, Indiana University, Bloomington, IN 47405 USA

We study genes necessary for DNA repair and meiosis in *Coprinus cinereus*, a basidiomycete in which the meiotic process is naturally synchronous. Using screens for mutants sensitive to ionizing radiation, we identified four genes (rad3, rad9, rad11 and rad12) necessary for both surviving irradiation and the completion of meiosis. The genes rad11 and rad12 encode the *C. cinereus* orthologs of Mre11 and Rad50, respectively; these proteins form a complex necessary for pathways of DNA double strand break repair. The rad9 gene encodes a large protein whose homologs have been shown to function in mitotic sister chromatid cohesion. To examine meiotic sister chromatid cohesion, we used B mating type locus-specific probes to differentially label homologs by fluorescence in situ hybridization. We found that Rad9 is necessary for sister chromatid cohesion during meiosis, and that about half the homolog pairing defects in the rad9-1 mutant are due to defects in meiotic sister chromatid cohesion. Using direct screens for meiotic mutants, we isolated the *C. cinereus* spo11-1 mutant, which is completely defective in meiotic chromosome synapsis and yet fairly proficient at homolog pairing. The spo11 gene encodes a topoisomerase-like protein shown in other systems to be required for the initiation of meiotic recombination. We found that the specific inhibition of premeiotic DNA replication (by use of the *C. cinereus* spo22 mutation) suppresses synapsis defects for both spo11-1 and for a rad50 mutant, rad50-4. Our results show that Spo11-induced recombination is not absolutely required for synapsis in *C. cinereus*, and that the early meiotic role of both Spo11 and Rad50 in synapsis partially depends on premeiotic S phase. This dependency likely reflects either a requirement for

these proteins imposed by the premeiotic replication process itself or a requirement for these proteins in synapsis when a sister chromatid (the outcome of DNA replication) is present.

Post-transcriptional gene silencing: a defense mechanism conserved in eukaryotes. Carlo Cogoni, Caterina Catalanotto, Gianluca Azzalin, Giuseppe Macino. Universita' di Roma, Genetica Molecolare, Rome, Italy

Post-transcriptional gene silencing (PTGS) as a consequence of the introduction of either DNA or RNA molecules, has been found to occur in a number of species. Trangenes can induce sequence-specific mRNA degradation in plants in a phenomenon termed co-suppression, in which the expression of both the introduced transgenes and the homologous endogenous genes were co-ordinately suppressed. A similar transgene-induced PTGS mechanism called quelling occurs in the fungus *Neurospora crassa.* Recently, it has been found that not only transgenes but also double-stranded RNA (dsRNA) molecules when injected into C. elegans specifically interfere with the expression of homologous resident genes. Analogous examples of dsRNA interference (RNAi) were subsequently documented in a number of invertebrate and vertebrate species. Although quelling, co-suppression and RNAi show consistent differences in the events that trigger PTGS, evident similarities have lead to the idea that the underlying molecular mechanisms could be related. Genetic dissection of the quelling phenomenon in *Neurospora crassa*, with the identification of quelling-deficient (qde) mutants and the isolation of the corresponding genes has paved the way for the identification cellular components of the PTGS machinery. Similar genetic approaches used in different systems such as *C. elegans* and *A. thaliana* have indeed demonstrated the existence of a common genetic base for PTGS. In the last year, these genetic approaches together with biochemical studies have produced a spectacular progress in the comprehension of PTGS mechanisms and a unified model for PTGS phenomena is now emerging.

Respiration controls mitochondrial DNA stability and longevity in *Podospora anserina*. Annie Sainsard-Chanet, Eric Dufour and Séverine Lorin. Centre de Génétique Moléculaire du CNRS, 91118 Gif sur Yvette, France.

In *Podospora anserina*, vegetative growth is systematically limited and the senescence process is always correlated with mitochondrial DNA instability. To date, the control of this process is poorly understood. We recently demonstrated that respiration plays a key role in this control. Inactivation of the nuclear *COX5* gene encoding subunitV of the cytochrome c oxidase complex and in consequence, the loss of the cytochromic pathway and the exclusive use of the alternative one, leads to a striking increase of longevity (> 30-fold for some subcultures) and to stabilization of the mitochondrial chromosome. We also showed that this respiratory modification leads to a decrease in ROS and energy production. In order to determine the respiratory parameters involved in the control of longevity, we decided to directly test whether the level of expression of the alternate oxidase, assumed to limit the production of ROS in plant mitochondria, affects lifespan in *P. anserina*. For this, we isolated and characterized the *AOX1* gene encoding the alternative oxidase. We demonstrated that both overexpression and disruption of this gene have no effect on mitochondrial DNA stability or longevity. We are now testing the effects of the overexpression of the alternative oxidase in long-lived cytochrome *c* oxidase

deficient mutants. Our preliminary results support the hypothesis that longevity is controlled by the global respiration efficiency and not by the expression level of the alternative oxidase.

Interdependency of chromosomal and cell cycle processes. Denise Zickler[1], Diana van Heemst[1,2], Etta Kaffer[3], Sophie Tessé[1], Gwenaël Ruprich-Robert[1], Marguerite Picard[1]. [1] Institut de Génétique et Microbiologie, Université Paris-Sud, Orsay, France
[2] Dept of Genetics, Agricultural University, Wageningen, The Netherlands. [3] Dept of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, B.C., Canada

SPO76/BIMD genes of *Sordaria macrospora* (1) and *Aspergillus nidulans* (2) encode a conserved protein which provides a molecular link between mitotic and meiotic chromosome morphogenesis: it localizes on chromosomes in analogous periods and both mutants exhibit closely related defects in chromosome morphogenesis in both divisions. In the absence of the protein, cohesion and compaction are coordinately affected (1). Moreover, such a transition occurs additionally at Sordaria meiotic midprophase, temporally correlated in wild-type cells with strong localization of Spo76p along the chromosome axes, synaptonemal complex (SC) formation and the loss of early recombination nodules (1). Spo76-GFPp allows direct visualization of chromosome behavior during synapsis in both wild-type and in mutants of the six genes, screened as suppressors of the sister cohesion defect of *spo76-1*.
Interestingly, while Spo76-GFP forms strong lines of continuous supra-axial staining during meiotic prophase in Sordaria (1), BIMD-GFP does not localize preferentially to the chromosome axes during the meiotic prophase of Aspergillus. This difference may be related to the absence of synaptonemal complex and crossover interference in *A. nidulans* (3, 4).
Spo76p and BIMD are functional homologues. Both mutants are sensitive to DNA-damaging agents and *bimD6* displays mitotic recombination defects. It reduces the level of mitotic interhomolog recombination but does not change the ratio between crossover and noncrossover outcomes and also is normal for intrachromosomal "gene conversion". *BIMD* also influences cell cycle progression: overexpression causes an arrest in G1-S (2) and, when compared to wild type, the rate of progression through G1/S/G2/M is increased in the *bimD6* mutant. Moreover, BIMD function is required during both G1/S and S/M phases.
Mutations in the *Podospora anserina* mitochondrial citrate synthase gene (*cit1*) reveal that the meiotic diffuse stage is likely a metabolic checkpoint for meiotic completion. This gene was identified as a suppressor of the metabolic defects of the peroxysomal assembly *car1/pex2* mutants (6). All mutants are viable, show wild-type respiration rates but higher catalase activity. All *cit1* mutations impair meiotic progression and most (including the null allele) block meiosis after synapsis, at the same diffuse stage when oocytes commonly stop for a long period of time.
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Cell Biology

1 **An IQGAP-related protein controls actin ring assembly and septation in *Ashbya gossypii***. Jorgen Wendland. Friedrich Schiller-University of Jena, Dept. of Microbiology.

The organization of the actin cytoskeleton is highly ordered in hyphae of filamentous fungi during polarized growth. Cortical actin is clustered in hyphal tips and actin cables serve as tip-directed tracks to promote vesicle delivery. Additionally, at sites of septation actin-ring structures occur. Actin-based contraction processes have been shown to be critical during cytokinesis of yeast-like and mammalian cells. In order to analyze these events in a filamentous fungus the *A. gossypii* homolog of an IQGAP-related protein encoded by the *Saccharomyces cerevisiae CYK1/IQG1* gene was isolated. The AgCyk1p encodes a protein of 1553 aa which is 53 aa longer than its yeast homolog. Both proteins are about 30% identical and share CH-domains (involved in actin filament binding), eight IQ-repeats (presumed to interact with calmodulin) and GAP-domains (GTPase activation). In addition, AgCyk1p possesses a PE-repeat domain which is absent in the yeast homolog. Deletion of *AgCYK1* produced viable mutant strains that grew with wt-like rates whereas in *S. cerevisiae CYK1* deletion results in lethality. *Agcyk1* mutants, however, showed an increased rate of lysed hyphae as well as a sporulation defect. Septation was compared between wild-type and *Agcyk1* mutants by fluorescence microscopy. The absence of actin-ring structures in these mutants was found to be accompanied by a lack of chitin accumulation at presumptive sites of septation. Our results indicate that *Agcyk1* controls actin-ring assembly and is also required to direct chitin deposition at sites of septation. In contrast to yeast-like fungi in filamentous ascomycetes polarization of the actin cytoskeleton in the growing hyphal tips and at sites of septum formation can occur at the same time and lack of septation does not interfere with polarized hyphal growth.

2 **Searching for additional components in the mating-type associated vegetative incompatibility response.** Megan D. Hiltz[1],Patrick K.-T. Shiu[2],N. Louise Glass[1]. [1]UC Berkeley, PMB, Berkeley CA USA. [2] Stanford University, Biology, Palo Alto CA

Vegetative incompatibility is a common and ubiquitous phenomenon in many filamentous fungi, from Ascomycetes to Basidiomycetes. In our lab, we use *Neurospora crassa* to characterize the components and mechanisms behind vegetative incompatibility to improve the understanding its function and its role as a non-self recognition system. In the vegetative incompatibility response, there is a prevention of vigorous heterokaryons, which is mediated when there is genetic dissimilarity between the fusing individuals at a heterokaryon incompatibility (*het*) locus. Incompatibility is noted in heterokaryons that exhibit growth inhibition, hyphal compartmentation and death. *A* and *a* represent the two mating-types (at the *mat* locus) in *N. crassa* required to fuse in the initiation of the sexual cycle. During vegetative phase, the *mat* locus also functions as one of the 11 *het* loci. The *tol* mutation causes a recessive suppression of mating-type vegetative incompatibility. While the molecular function of TOL is not apparent, it contains putative protein-protein interaction domains.
To determine the role of TOL in mating-type vegetative incompatibility and to identify other proteins involved in the process, TOL was used as bait in a yeast 2-hybrid system. Of the identified positive cDNA clones, one was characterized to deduce its role in mating-type associated vegetative incompatibility. *Ncvip1* has homology with *vip1*,which was identified in a p53 expression library screen of *Schizosaccharomyces pombe*. RIP (repeat-induced point) mutational analyses of the *Ncvip1* was attempted and the progeny from the cross examined as to their effects on mating-type associated vegetative incompatibility.

3 **Molecular genetic basis of resistance to the DMI fungicide in *Tapesia yallundae*.** Henry M. Wood, Paul S Dyer, Matt J Dickinson, John A Lucas*. University of Nottingham, Life & Environmental Sci. Nottingham, , UK. * IACR Long Ashton, Bristol UK

Resistance to DMI fungicides in fungal plant pathogens has previously been correlated with point mutations in the *CYP51* gene encoding the target enzyme eburicol 14alpha-demethylase. The *CYP51* gene was isolated from a genomic library of the cereal eyespot pathogen *Tapesia yallundae* using a PCR-based strategy with degenerate primers. The gene has a coding region of 1708 bp, containing two putative introns, and a predicted 526 amino acid polypeptide product which exhibits 55 - 68 % homology to *CYP51*s from other filamentous fungi. The *CYP51* gene was fully sequenced from four further field isolates of *T. yallundae* either sensitive or resistant to the DMI fungicide prochloraz. *CYP51* was also partially sequenced from two additional field isolates and eight progeny from a cross between prochloraz sensitive and resistant parents. Two allelic forms of the gene were detected termed *CYP51-1* and *CYP51-2*. These differed by 0.7 % over the coding region and exhibited differences in sequence upstream of the putative start codon. No correlation was found between change in sequence and fungicide sensitivity, with *CYP51-1* and *CYP51-2* alleles found in both sensitive and resistant isolates. Thus, contrary to frequent reports for resistance to other DMI fungicides, resistance to prochloraz in the isolates of *T. yallundae* examined in this study involved some other mechanism than mutation in the gene encoding the target site enzyme. There was also no evidence of increased copy number of *CYP51* as a possible basis of prochloraz resistance in *T. yallundae*

4 **Symmetry of branching in *Neurospora crassa***. Michael K. Watters. Valparaiso University, Valparaiso Indiana.

In a previous study of branching in *Neurospora*, it was determined that branching at the tip is not a tip autonomous process, but that branching is controlled at least in part by factors at or near the previous branch point. This was determined by the demonstration of a statistical correlation between lengths of branch intervals (the distance between two tandem branch points) having a common origin. That study was unable to determine the nature of that common factor. Namely, the correlation could have been due to the physical bisection of some structure or resource (such as the Spitzenkörper) at the time the common branch event occurred. It could have also been caused by the division, at the common branch point, of the flow of some factor (such as tip growth vesicles) toward the growing tips. This study seeks to distinguish between these two alternatives by extending the examination of branch interval correlation and comparing branch intervals which share a common origin, one step removed. The observation of branch length correlation at this level demonstrates that the previously observed correlation results from the division of some factor flowing toward the tip and not the singular division of some structure at the time of branch formation.

5 **The extracellular soluble polysaccharide (ESP) from *Aspergillus kawachii* improves the stability of extracellular enzymes and is involved in their localization.** Kazuhiro Iwashita, Nao Harada, Hitoshi Shimoi, Kiyosi Ito. National Research Institute of Brewing, 3-7-1, Kagamiyama, Higashihiroshima, Hiroshima, 739-0046, Japan

Aspergillus kawachii produces two extracellular beta-glucosidases (EX-1 and EX-2) and one cell wall-bound (CB-1) beta-glucosidase, which are derived from the same *bglA* gene. Extracellular beta-glucosidases (EX-1 and EX- 2) are quite stable in crude solution, but they become unstable in purified form under moderate condition. Purified extracellular beta-glucosidases bind to a cell wall fraction from mycelia, even though these enzymes are released into the medium under solid culture conditions. *A. kawachii* produces an extracellular soluble polysaccharide (ESP) under solid culture conditions. Addition of purified ESP remarkably stabilized the beta-glucosidases. ESP directly interacted with the purified extracellular beta-glucosidases but it did not affect the *Km* value of these enzymes. Moreover, ESP inhibited the adsorption of purified extracellular beta-glucosidases to the cell wall fraction and extracted them from it. To examine the effect of ESP to other extracellular enzyme, we purified extracellular alpha-glucosidase and alpha- galactosidase of *A. kawachii*. As expected, ESP stabilized these two enzymes and inhibited their adsorption to the cell wall fraction. These results indicate that ESP plays an important role in the stability and localization of extracellular enzymes. ESP from *A. kawachii* directly binds to the enzymes and releases them to the medium from the cell wall layer and then stabilizes them.

6 **Cell cycle progression and cell polarity require sphingolipid biosynthesis in fungi.** Jijun Cheng, Anthony Fischl and Xiang S. Ye. Infectious Diseases Research, Lilly Research Laboratory, Eli Lilly and Company, Indianapolis, IN 46285

Sphingolipids are major components of the plasma membrane of eukaryotic cells but their physiological functions are not well understood. Here we show that sphingolipid biosynthesis is required for cell cycle progression in G1 and cell polarity in *Aspergillus nidulans*. Genetic or pharmacological inactivation of *aurA* encoding inositol

phosphorylceramide (IPC) synthase causes cell cycle arrest in G1 and also prevents polarized hyphal growth of germinating spores. Inactivation of IPC synthase eliminates IPC synthesis but also leads to the accumulation of its upstream intermediate, ceramide. Serine palmitoyl CoA transferase (SPT) is the first committed step of the sphingolipid biosynthesis pathway. Inactivation of SPT also prevents polarized growth but not nuclear division of germinating spores, indicating that IPC is required for cell polarity and that accumulation of ceramide causes G1 arrest. Interestingly, inhibition of sphingolipid biosynthesis in germlings promotes rapid multiple branching at the hyphal tip, which normally never occurs. Hyphal tip branching is closely associated with a dramatic rearrangement of the actin cytoskeleton and is dependent on the actin function. Sphingolipids have no effect on the microtubule. The results indicate that sphingolipids regulate fungal cell polarity via the function of actin cytoskeleton.

7 **Visualization of nuclei in *Aspergillus oryzae* by expressing EGFP.** Jun-ichi Maruyama, Harushi Nakajima, and Katsuhiko Kitamoto. Department of Biotechnology, The University of Tokyo, Tokyo, Japan.

Aspergillus oryzae is an important fungus in the fermentative industry and has been used in sake, soy sauce and *miso* manufacture as well as production of commercial enzymes. In spite of its fermentative and industrial use little is known about the cellular and developmental processes. Nuclear migration is one of the most important processes because *A. oryzae* forms multinucleate cells in conidia as well as hyphae. We focused on nuclear migration in *A. oryzae*. In order to stain nuclei in *A. oryzae*, we fused *A. nidulans* histone H2B with EGFP. The fusion protein, H2B::EGFP, successfully visualized nuclei in hyphae and conidia. Time-lapse observation exhibited that apical nuclei migrated toward tips of growing hyphae. In a conidium varied numbers of nuclei, ranging from one to three or four, were observed. FACS analysis also supported this result. In filamentous fungi cytoplasmic dynein and dynactin complex are required for nuclear migration. We have cloned *arpA* and *dhcA* genes, encoding Arp1 in dynactin complex and cytoplasmic dynein heavy chain, respectively, and analyzed phenotypes of these disruptants. Time-lapse observation by expressing H2B::EGFP revealed that both *arpA* and *dhcA* disruptants showed a defect in nuclear migration.

8 **Determinants of fungal morphogenesis: isolation and analysis of *Neurospora crassa* mutants defective in the maintenance and regulation of cellular polarity.** Stephan Seiler and Michael Plamann. UMKC, 5100 Rockhill Road, Kansas City, MO 64110

Elongating at rates up to a micrometer per second, filamentous fungi are among the fastest growing cells known and are a good system to study cellular polarity. By filtration enrichment and a colony overlay method, we have isolated a large collection of temperature sensitive mutants in Neurospora crassa defective in the maintenance and regulation of growth polarity as a basis to study cellular developement in filamentous fungi. Phenotypical analysis and complementation tests of ca 800 mutants have allowed us to identify 20 morphological classes that define more than 80 genes involved in polar or directed growth. The observed phenotypes of polarity mutants range from a total loss of polarity over the whole hypha, generating chains of spherical cells, to more specific defects localized to hyphal tips or to subapical regions. These mutant phenotypes suggest that fungal morphogenesis is the result of the well balanced action of proteins at the tip as well as subapically to produce and maintain the characteristic tube-like structure of the hypha. Additional mutants, defective in the regulation of polarity and growth directionality, exhibit abnormal side branches and highly irregular growing hyphae, implying defects in the establishment of mature tips and Spitzenkörper positioning, respectively. Currently we are isolating and sequencing genes defined by representative mutants of each phenotypic class to understand tip growth and polarity in filamentous fungi

9 **Unraveling G-protein mediated signaling in *Phytophtora infestans.*** Maita Latijnhouwers[1], Ana Laxalt[2], Teun Munnik[2], and Francine Govers[1]. [1]Wageningen University, Phytopathology, Wageningen, Netherlands. [2]University of Amsterdam, Swammerdam institute, Amsterdam, Netherlands

Many fungal plant pathogens develop highly specialized infection structures in response to signals detected upon contact with the plant. In addition, proliferation within host tissue requires both metabolic and morphological adaptation to the plant environment. Work on several plant-pathogenic fungi revealed a role for G-protein- and cAMP-mediated signal transduction in these processes. The general finding is that mutants with defects in components of the cAMP-signaling pathway have attenuated virulence. Our aim is to study the role of G-protein signaling in pathogenesis of the oomycete *Phytophthora infestans*, the causal agent of potato late blight. We isolated and characterized *P. infestans* genes coding for Galpha (*Pigpa1*) and Gbeta (*Pigpb1*) protein subunits, with up to

42% and 62% identity with known G-protein subunits in the database, respectively. Expression studies showed that both genes are differentially expressed in various stages of the life cycle. In an EST database, clones encoding putative catalytic and regulatory subunits of cAMP-dependent protein kinase (PKA) were selected. Through homology dependent gene silencing PIGPA1 and PIGPB1 deficient transformants will be obtained and this will enable us to determine the function of *Pigpa1* and *Pigpb1* in *P.infestans*. Phospholipid signaling was investigated by treating sporangiospores with the G-protein activator mastoparan. This treatment resulted in increased levels of the phospholipids PA and DGPP, as was detected using TLC analysis. This shows that mastoparan activates PLD in *P. infestans* and strongly suggests that in *P. infestans*, phospholipid and G-protein signaling are linked. PLC activation was not detected.

10 **Microtubules in the fungal pathogen *Ustilago maydis* are highly dynamic and determine cell polarity.** Irene Schulz[1], Roland Wedlich-Söldner[1], Marianne Brill[2], Gero Steinberg[1]. [1]MPI Terrestrial Microbiology, Marburg, Germany. [2]LMU Institut für Med. Mikrobi, Munich, Germany

Many fungal pathogens undergo a yeast-hyphal transition during their pathogenic development that requires the rearrangement of the cytoskeleton, followed by directed membrane traffic towards the growth region. The role of microtubules and their dynamic behavior during this process is not well understood. Here we set out to elucidate the organization, cellular role and in vivo dynamics of microtubules in the dimorphic phytopathogen *Ustilago maydis*. Hyphae and unbudded yeast-like cells of *U. maydis* contain bundles of spindle pole body-independent microtubules. At the onset of bud formation two spherical tubulin structures focus microtubules towards the growth region, suggesting that they support polar growth in G2, while spindle pole body-nucleated astral microtubules participate in nuclear migration in M and early G1. Conditional mutants of an essential a-tubulin gene from *U. maydis*, tub1, confirmed a role of interphase microtubules in determination of cell polarity and growth. Observation of GFP-Tub1 fusion protein revealed that spindle pole body-independent and astral microtubules are dynamic with elongation and shrinking rates comparable to those found in vertebrate systems. In addition, very fast depolymerization was measured within microtubule bundles. Unexpectedly, interphase microtubules underwent bending and rapid translocations within the cell suggesting that unknown motor activities participate in microtubule organization in *U. maydis*.

11 **Isolation and characterization of novel Ran-binding proteins in the yeast *Saccharomyces cerevisiae.*** Andreas Braunwarth[1], Thomas Gerstberger[1], Micheline Fromont-Racine[2], Pierre Legrain[2], Ed Hurt[1] and Markus Kunzler[1]. [1]Biochemie-Zentrum Heidelberg (BZH), Ruprecht-Karls-Universitat, Im Neuenheimer Feld 328, 4. OG, D-69120 Heidelberg, Germany; [2]Institut Pasteur, Genetique des Interactions Macro-moleculaires, Departement des Biotechnologies, 25-28 rue du Docteur Roux, F-75724 Paris cedex 15, France

The Ran GTPase switch plays a key role in nucleocytoplasmic transport and, as shown more recently, in the formation of microtubule asters and the nuclear envelope. Despite the apparent functional diversity of this GTPase only a limited number of Ran-targets is known mostly implicated in nucleocytoplasmic transport. We applied a 2-hybrid approach in order to identify novel Ran-binding proteins in the yeast *Saccharomyces cerevisiae*. As baits we used both wildtype Ran, Gsp1, as well as two mutant forms, Gsp1(G21V) and Gsp1(T26N), which are locked in the GTP- or GDP-bound form, respectively. We will present the results of these screens by which we identified known Ran-binding proteins, such as Ran-binding protein 1 (Yrb1), NTF2, Mog1 and various members of the beta-karyopherin family, as well as a number of uncharacterized ORFs which are candidates for novel Ran- binding proteins. We will present the characterization of one of these ORFs, termed Yrb30, which was identified as the only prey besides Yrb1 in the Gsp1(G21V) screen. Consistent with its way of isolation, we could demonstrate that Yrb30 binds, like Yrb1, specifically to the GTP-bound form of Gsp1. This was shown both by *in vitro* pull-down experiments using recombinant proteins as well as by copurification of the proteins from yeast. Interestingly, Yrb30 localizes, like Yrb1, to the cytoplasm. In contrast to Yrb1, however, which acts as a coactivator of RanGAP, Yrb30 inhibits RanGAP-mediated GTP-hydrolysis by Ran. We are currently trying to understand the cellular function of this novel RanGTP- binding protein.

12 **Molecular mechanism of resistance to terbinafine: cloning of a fragment that turns *Aspergillus nidulans* resistant to this antifungal agent.** Graminha, M.A.S.; Rocha, E.M.F. and Martinez-Rossi, N.M.. Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil.

The allylamine antimicotic agent terbinafine is employed both orally and topically for the treatment of fungal infections of the skin, nails and hair. Its mechanism of action is related to a specific inhibition of squalene epoxidase, an enzymatic step essential in the synthesis of ergosterol, resulting in deficiency and accumulation of intracellular squalene that leads to cell death. Little is known about the molecular mechanisms involved in the resistance to this antifungal agent. Thus, in order to understand the mechanisms involved in resistance to terbinafine we studied resistant mutants isolated in our laboratory by UV light irradiation. Genetic analysis revealed that a co-dominant gene located on chromosome IV is responsible for resistance. In an attempt to clone this gene we constructed a mutant genomic library and isolated a clone carrying a 6.5 kbp fragment that complements a sensitive GR5 strain by sib selection. After subcloning, a 3.5 kbp fragment still had the ability to make GR5 resistant. The clone was sequenced and Blast analysis revealed the presence of an entire ORF (accession number AF316427) homologous to the *Pseudomonas putida* salicylate 1- monooxygenase gene and part of an ORF homologous to the *Candida albicans* fatty acid synthase beta subunit. We are currently attempting to define which of these two genes is involved in terbinafine resistance. Financial support: FAPESP, CNPq and CAPES

13 **The peroxisome biogenesis gene *PEX6* of*Colletotrichum lagenarium* is required for appressorium-mediated plant infection.** Akiko Kimura, Yoshitaka Takano, Iwao Furusawa, and Tetsuro Okuno. Kyoto University, Graduate School of Agriculture, Kyoto, Japan.

Restriction enzyme-mediated integration (REMI) mutagenesis of *Colletotrichum lagenarium*, the causal agent of cucumber anthracnose, led to the identification of a pathogenicity gene *ClaPEX6*. The *ClaPEX6* gene encodes a protein showing high homology to Pex6 proteins involved in peroxisome biogenesis. *clapex6* mutants, generated by gene replacement, lacked pathogenicity to the host plant whereas they showed normal growth and conidiation on nutrient-rich medium. Conidia of *clapex6* mutants germinated and formed appressoria which were smaller than those of the wild type and which showed severe reduction of melanization. The appressoria of the mutants failed to form penetration hyphae into the host plant.The *clapex6* mutants caused lesion formation when inoculated through wound sites. These results indicate that the pathogenicity defect of *clapex6* mutants is due to formation of appressoria defective in penetration. To investigate whether *clapex6* mutants show impaired peroxisome biogenesis, the green fluorescent protein (GFP) containing a peroxisome targeting signal type 1 (PTS1), designated GFP-PTS1, was expressed in both wild type and *clapex6* mutant. In the *clapex6* mutant, the GFP-PTS1 proteins showed no specific localization whereas they localized in peroxisomes in the wild type. *clapex6* mutants failed to grow on oleic acid as the sole carbon source, which indicates a defect in fatty acid beta-oxidation in peroxisomes. Our results show that metabolic functions of peroxisomes are necessary for appressorium-mediated infection by *C. lagenarium*.

14 **Branching of *Achlya ambisexualis* hyphae results in concomitant alterations in the levels of heat-shock protein chaperones.** Julie C. Silver, Lianna Kyriakopoulou, Chai Chen and Shelley A. Brunt. Department of Medical Genetics and Microbiology and Division of Life Sciences, University of Toronto at Scarborough, Toronto, Ontario, Canada M1C 1A4.

Heat shock protein chaperones, assist in protein folding, oligomerization and translocation in the cytoplasm, nucleus, mitochondria and endoplasmic reticulum in all eukaryotic cells. In addition, the chaperones Hsp90 and Hsp70 along with specific other chaperones and co chaperones, are essential for maintaining high level ligand binding ability by steroid receptors and for the delivery of these receptors to the nucleus. Basal levels of the *Achlya* heat shock protein chaperones Hsp90, Hsp70-1, cytoplasmic Hsp70, mitochondrial Hsp70 (Ssc1), endoplasmic reticulum Hsp70 (Bip/Kar2/Grp78) and mitochondrial Hsp60 (GroEL), are present in tip-growing vegetative hyphae of *A.ambisexualis*. However, with the exception of one of the two tunicamycin and calcium ionophore- inducible Hsp70 proteins (Hsp70-1) which decreased, the levels of each of the above chaperones and of their respective mRNAs, increased significantly when the normally smooth and unbranched hyphae, develop numerous lateral branches as a result of treatment with the *Achlya* steroid hormone antheridiol. Very similar changes in the chaperone transcript levels tested were observed when branching was induced by casein hydrolysate, even though the casein-induced branches have a different morphology and function than antheridiol-induced branches. Nuclear run-on assays demonstrated that the transcription of genes encoding at least three of the above chaperones, was increased in antheridiol- treated cells. Supported by grants to J.C.S from NSERC Canada.

15 **Characterization of the cAMP signalling pathway in *Aspergillus nidulans* and its role in conidiospore germination**. S. Fillinger, M.-K. Chaveroche, and C. d'Enfert. Unite de Physiologie Cellulaire, Institut Pasteur/Paris, France

In the yeast *Saccharomyces cerevisiae* cAMP signalling is relatively well understood; its implication in such diverse cellular processes like differentiation, mating, stress response or nutrient sensing has been proven. In filamentous fungi cAMP signalling has been involved in developmental processes, therefore contributing to the virulence of pathogenic fungi. However a holistic view of cAMP signalling in filamentous fungi is still missing. We have choosen the model fungus *A. nidulans* to rapidly characterize the elements involved in cAMP signalling and to establish a first interaction network. We have cloned and inactivated the major elements of this signalling cascade: the adenylate cyclase gene, *cya*, the gene encoding the regulatory subunit of protein kinase A, *pkaR*, and the gene encoding another protein kinase of type A, *schA*. Using genetic and phenotypic analyses we have investigated the role of CyaA, SchA, PkaC (K. Shimizu and N. Keller/Texas A&M), and Ras during spore germination and colony establishment. In particular, we have monitored in single and double mutants the kinetics of trehalose breakdown which is a landmark of early stages of spore germination and the kinetics of germtube formation. Our results show that 1/ PkaC and SchA act synergistically to control trehalose breakdown and spore germination; 2/ cAMP synthesis is required for these two processes as well as proper colony development but that PkaC activity is also regulated by other signals; 3/ the small GTP-binding protein Ras appears to be involved in the control of spore germination independently of cAMP synthesis. A first model for cAMP signalling in *A. nidulans* will be presented.

16 **The *Aspergillus nidulans* septin AspB localizes to areas of new growth pre- and post- mitotically.** Patrick J. Westfall and Michelle Momany. University of Georgia, Department of Botany, Athens, GA.

The septin family of proteins acts as an organizational scaffolding in areas of cell division and new growth. In the filamentous fungus, Aspergillus nidulans, the septin encoding gene, aspB, produces protein that localizes to areas of cellular division and new growth both pre and post-mitotically. AspB localizes at the septum post-mitotically with an underlying polarity evident as cytokinesis progresses. This localization at the septum is dependent on actin, and occurs before the crosswall starts to form. AspB also localizes to areas of new growth including secondary germ tubes and branches. AspB localizes pre- mitotically as a ring at sites of secondary germ tube emergence and branching and is the only known branch site marker. Localization to the secondary germ tube and new branch points is transient, and disappears as the hypha extends. In addition, AspB is found at several stages during the development of the asexual reproductive structure, the conidiophore. It localizes transiently to the vesicle/metulae and metulae/phialide interface, and persistently to the phialide/conidiospore interface.

17 **Cloning of the *Aspergillus nidulans lip*A gene, where mutation confers resistance to undecanoic acid.** Ana G. Brito[1], Ana L. Fachin[2], Monica S. Ferreira-Nozawa[2], Nilce M. Martinez-Rossi[2] and Antonio Rossi[1]. [1]Departamento de Bioquímica e Imunologia, [2]Departamento de Genética. Faculdade de Medicina de Ribeirao Preto, Universidade de São Paulo, Brazil

Undecanoic acid may affect respiration, pigment formation or fatty acid synthesis in some fatty acid-sensitive fungi. *Trichophyton rubrum*, a fungus causing superficial mycoses in skin, hair and nails, depends on the secretion of some enzymes (lipases, keratinases, etc.) to parasitize its host, since these enzymes have not been observed in non-pathogenic strains (uda^r). Furthermore, these strains, in contrast to the pathogenic ones (uda^s), are resistant to undecanoic acid. We selected mutants resistant to undecanoic acid from both *A. nidulans* and *T. rubrum*, and two resistant *A. nidulans* mutants (designated *lip*A1 and *lip*A2) were mapped on chromosome VIII and showed a dominant character. A genomic library of *lip*A1 has been constructed in plasmid pUC18 (*Bam*HI/BAP) and used to transform sensitive *A. nidulans* strains. The clone containing the gene for resistance to undecanoic acid was recovered from *E. coli* transformants and sequenced (accession number AF315651) and its aligment with sequences of GenBank revealed homology to triacylglicerol lipases, indicating that this enzyme could be a determinant of fungal pathogenicity. Financial Support: FAPESP, CAPES, CNPq and FAEPA.

18 **The role of *Aspergillus nidulans scaA* gene on S and G2 mitotic checkpoints induced by DNA damage.** Renata Castiglioni Pascon, Marcia Regina von Zeska Kress Fagundes, Marcelo A. Vallim, Maria Helena S. Goldman[1] and Gustavo H. Goldman. Faculdade de Ciencias Farmaceuticas de Ribeirao Preto and [1]Faculdade de

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The DNA damage response in *A. nidulans* is mediated through cdc2 tyrosine 15 phosphorylation. Several other genes have been shown to play a role in DNA damage induced checkpoints, as *uvsB* (ATM kinase homologue) and *uvsD* (Rad 26 homologue). Mutation in these genes cause premature mitosis in the presence of damaged and unrepaired DNA. The *Aspergillus nidulans scaA* gene was initially isolated as a genetic determinant for camptothecin resistance, an anti-tumor drug known to stabilize the DNA/Topoisomerase I complex. The cloning and sequencing of this gene showed that the protein has moderate similarity with the human nibrin gene. This protein is the target of the ATM Kinase and forms a protein complex with Rad50 and Mre11 in higher eukaryotes. In humans, when this gene is mutated, it causes the Nijmegen Breakage Syndrome. Here, we show that *A. nidulans scaA* gene is necessary not only for camptothecin resistance,but also to respond to a large range of DNA damage agents, such as 4-nitroquinoline oxide, UV-light, gamma-rays, berberine, the DNA cross-linking agent mitomycin C, and the DNA replication blocking agent, hydroxyurea. The *scaA* mutant strain does not undergo mitotic delay in the presence of DNA damage as the wild type. Actually, it acts similarly to a cdc2AF strain (FRY 20), suggesting that this protein has a role in G2/M checkpoint control. Surprisingly, a S-phase delay that is caused by 6 mM of hydroxyurea activates a S/G2 checkpoint, impairing development of germ tubes and aberrant nuclear morphology and decreased viability in the disrupted *scaA* strain. This suggests that *scaA* operates a very important function in S-phase, however is not part of a checkpoint in this stage of the cell cycle. Financial Support: FAPESP and CNPq, Brazil.

19 **Genetic analysis of cytokinesis mutants in *Ustilago maydis.*** Leonora Leveleki and Michael Bölker. University of Marburg, Dept. of Biology, Karl-von-Frisch-Strasse, D-35032 Marburg

The main interest of our group is the study of morphological and cytokinesis mutants in the dimorphic basidiomycete *Ustilago maydis.* The cells of the don mutants (*don1* and *don3*), which have been investigated in our laboratory, are not able to form a secondary septum, which is required for cell separation. Thus, these cells stay together and form tree-like structures.
Don1 and Don3 play a role in a GTPase cycle. Don1 acts as a guanine nucleotide exchange factor specific for GTPases of the Rho/Rac family and activates Cdc42. Active Cdc42 is supposed to activate the Don3 protein, a Ste20-like protein kinase, which presumably effects a protein kinase cascade. We are currently investigating the role of this signalling cascade for cell separation.
To identify additional genes, that play a role in the same pathway upstream or downstream of *don1* and *don3*, we performed a genetic screen to isolate additional mutants with the same, tree-like phenotype. By UV irradiation, 51 don-like mutants could be isolated. Complementation analysis of these mutants demonstrated that 40 of them carry the *don1* mutation, none of them the *don3* mutation and 11 of them carry mutations in novel genes.
We have complemented some of these mutants by transformation with a *U. maydis* cosmid library. Restriction mapping and subcloning of the cosmids were carried out to analyze the newly identified genes in more detail.
The cytokinesis defect in the mutant cells was further characterized by localizing the septin homolog Cdc10 as a GFP fusion protein. In addition, the mutants were analyzed by immunostaining and confocal laser scanning microscopy (CLSM) for the structures of the septum and the microtubule cytoskeleton, and the localization of endosomal vesicles which are supposed to be involved in cell separation.

20 **The snxA suppressor of the *Aspergillus nidulans* nimX2 mutation affects S-phase checkpoint control.** Sarah Lea McGuire, Ann Long, Allison McElvaine. Milsaps College Biology Department, Jackson MS

We have recently isolated extragenic suppressors of the *Aspergillus nidulans nimX2*^{cdc2} mutation, designated *snxA-snxD*, for *s*uppressors of *n*im*X* (see McGuire et al., *Genetics* 156, December 2000). The *snxA1* mutation is allele-specific, causes cold-sensititivity and leads to aberrant nuclei when conidia are germinated at 20C. Reciprocal shift experiments suggest that *snxA1* leads to arrest of nuclear division during G_1 or early S at the restrictive temperature. The *snxA1* mutation is pleiotropic in that in addition to causing nuclear division defects at 20C, the mutation allows cells to divide in the absence of a normally functional NIMX^{CDC2} (e.g., it was isolated as a suppressor of *nimX2*). In order to better understand the interactions of SNXA with NIMX^{CDC2}, we are analyzing the effects of two drugs which affect checkpoint control, hydroxyurea (HU) and methylmethanesulfonate (MMS), on *snxA1/nimX2* double mutants at 42C (the restrictive temperature for *nimX2*). In the presence of 10mM HU at 42C, 58% of *snxA1/nimX2* cells enter a lethal premature mitosis, compared to 0% of either single mutant or wild type cells. In addition, in the

presence of 0.02% MMS at 42C, 62% of *snxA1/nimX2* cells enter premature lethal mitosis, compared to 0% or 3% of wild type cells or single mutants, respectively. We are in the process of analyzing $NIMX^{CDC2}$ activity and tyrosine-15 phosphorylation of $NIMX^{CDC2}$ in single and double mutants under various conditions to determine the effects of the *snxA1* mutation on these. These data suggest that *snxA1* affects S-phase checkpoint control of $NIMX^{CDC2}$. *Supported by NIH grant R15GM55885*.

21 **Circadian rhythms in *Neurospora crassa*: A new way to study rhythms in frq-less strains.** Tabitha Granshaw and Stuart Brody. Biology Dept. UCSD, La Jolla, CA.

The frq gene is an important component of the Neurospora clock. A strain carrying frq10, a deletion of this gene, expressed a conidiation rhythm (banding) only after 5 to 7 days of growth. Although it had a period of 24 to 27 hours at 20C, the banding was not very robust or synchronous, making it difficult to study the frq-less rhythm. To circumvent these problems, a set of culture conditions was developed to produce synchronous, robust and reproducible banding in Petri dishes. A key aspect of the improved culture conditions is the addition of trans-farnesol or geraniol, both of which are related to intermediates in the steroid pathway. The addition of trans-farnesol, at a final concentration of 5x10-5 M, produced a period between 23 and 26 hours, whereas geraniol, at a final concentration of 40x10-5 M, lowered the period to between 17 and 21 hours. The growth rate was reduced 30% by trans-farnesol and 20% by geraniol. Other properties of the circadian rhythm missing in frq10, i.e. light sensitivity and temperature compensation, were not restored when frq10 was grown on geraniol or trans-farnesol. Neither chemical altered the period (21 hours) of the control strain, bdcsp, but the growth rate was inhibited 60% by trans-farnesol and 40% by geraniol. The existence of some part of the circadian system remaining in frq10 has lead to the idea of a two-oscillator system, composed of the frq/wc oscillator and the frq-less oscillator. The first part of the system is presumably disabled by the loss of the frq gene. Since frq10 bands robustly and synchronously on both trans-farnesol and geraniol, the use of these culture conditions could potentially allow the study of the "frq-less" oscillator.

22 **Rho GTPases in *Aspergillus nidulans*.** Gretel M. Guest and Michelle Momany. Department of Botany, University of Georgia Athens, GA

Rho GTPases are enzymes that link extracellular growth signals or intracellular stimuli to the assembly and organization of the actin cytoskeleton. They function as binary switches that cycle between the active GTP-bound form and inactive GDP-bound form. Rho GTPases have been studied in a variety of systems including Saccharomyces, Fucus, Dictyostelium, and human neutrophils. In *S. cerevisiae* the Rho GTPases establish and maintain polarity by affecting downstream targets which influence actin organization. Little is know about genes controlling polar growth of filamentous fungi. Actin undergoes constant reorganization especially at the tip of a growing hypha. The signals triggering this are unknown, but given the Rho GTPases function in other organisms, it seems likely that they may play a part in the actin reorganization process in filamentous fungi. In an effort to identify the possible role of Rho GTPases in *A. nidulans*, a 400 bp region of RHO 1 and RHO 3 from *S. cerevisiae* was used to identify the putative homologs from an *A. nidulans* cosmid library. rho1 and rho3 have been identified as being on chromosomes 5 and 6, respectively. Currently sequencing, targeted gene replacement, and cytological studies are underway to understand the function of these genes.

23 **A domain in Tom40 required for assembly and stability of the TOM complex in *Neurospora crassa*.** Rebecca D. Taylor and Frank E. Nargang. University of Alberta, Biological Sciences, Edmonton, Alberta, Canada.

Mitochondria are nearly ubiquitous in eukaryotes and are the site of many important biological functions. Most proteins required by mitochondria are encoded by nuclear genes and are synthesized on free cytosolic ribosomes before being imported into mitochondria. The TOM complex (translocase of the outer membrane) is responsible for initial recognition and translocation of mitochondria targeted precursor proteins across the outer mitochondrial membrane. Recognition of preproteins is mediated by the receptors of the TOM complex, Tom20, Tom22 and Tom70. Tom40, the major component of the TOM complex, is thought to form a pore through which preproteins traverse the outer membrane. Alignment of Tom40 from various species reveals a conserved domain in the amino terminus of the protein that is thought to be found in the intermembrane space of mitochondria. We have made specific mutations in this region via site directed in vitro mutagenesis. Our results suggest that this conserved amino

terminal domain is required for assembly of Tom40 into the TOM complex, or required to maintain the stability of Tom40 within the complex, or both.

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24 **Fungal cell biology profiling technology for high throughput functional genomics analysis in plant pathogenic fungi.** Sanjoy K. Mahanty, Matthew Tanzer, and Jeffrey R. Shuster. Department of Microbial Phenomics , Paradigm Genetics Inc., 108 Alexander Drive, RTP, NC 27709

At Paradigm Genetics Incorporated, the Microbial Research Group is obtaining information about the interplay between a microorganism and its internal and external enviornment through gene function analysis (using TAG-KOTM technology) for antifungal / fungicide discovery. Towards this end, we have developed a sophisticated cell biology profiling (CBP) platform. Our initial investigation is initiated by generating signatures through cell biology profiling technology in the budding yeast Saccharomyces cerevisiae. Cell Biology Profiling through microscopy plays a key role in defining specific targets affecting cell cytoskeleton, cell physiology and cell cycle. Microscopic images of various cell biological structures (actin, microtubule cytoskeleton structure, vacuolar, nuclear, mitochondrial and cell wall structure), data visualization, and quantitation provide the best understanding of the cellular processes and have greater impact on target discovery. CBP is the only technology that provides information on multiple interacting or independent components within or between living cells. The information enables more qualified new chemical entities to come out of the early drug discovery pipeline. Here, the microscopic images of various cell biological structures of plant pathogenic fungi, *Magnaporthe grisea*, *Mycosphearella graminicola* and *Botrytis cinerea* will be presented. Results with cell biology profiling (CBP) in discovery of novel and known cell biological targets, as well as the power of cell biology profiling in defining gene modulation studies with filamentous fungi will be presented.

25 **Stress-induced calcium responses in *Aspergillus nidulans*.** Diana C. Bartelt, Vilma Greene, and Adrienne Dolberry. Dept. of Biol. Sci., St. John's University, Jamaica, NY11439.

We have examined the response of the filamentous fungus *Aspergillus nidulans* to conditions of environmental stress using strains expressing apoaequorin in the cytoplasm and mitochondria to monitor intracellular [Ca2+] *in vivo*. Exposure to acute hypertonic, cold, and oxidative stresses elicit transient increases in cytoplasmic [Ca2+]. Hyperosmotic conditions brought about by the addition of osmotica such sorbitol, glycerol, glucose, KCl and NaCl at a concentration of 0.2 M cause a rapid transient in cytoplasmic [Ca2+] but have little effect on the mitochondrial Ca2+ pool. The response requires the presence of Ca2+ in the external medium and is unchanged with increasing osmotic strength. Exposure to hypotonic conditons has no effect on cytoplasmic [Ca2+]. Cold shock causes a prolonged elevation of cytoplasmic [Ca2+] which is not dependent upon the presence of Ca2+ in the external medium but is dependent upon prior exposure to calcium. All of the above responses can be inhibited by the presence of 5F-BAPTA in the external medium suggesting that, at least in part, the source of Ca2+ is extracellular. Oxidative stress induced by exposure to 10mM H2O2 causes an increase in cytoplasmic [Ca2+] without prior exposure of cells to calcium and an even greater increase in mitochondrial [Ca2+], neither of which is affected by the presence of 5F-BAPTA. Resting levels of [Ca2+] have been determined to be below the level of detection, (50 nM). Exposure to Ca2+ causes a dose-dependent transient increase in cytoplasmic [Ca2+]. The duration, but not the onset of this response is temperature-dependent. Supported by N.I.G.M.S. R15GM52630 and an I.M.S.D. grant to St. John's University.

26 **Isolation of mutants of *Aspergillus nidulans* showing hypersensitivity to calcofluor white.** Terry W. Hill[1], Darlene M. Loprete[1], Midu Bagrodia[1], Amit Mirchandani[1], Jennifer A. Livesay[1], and Michelle Momany[2]. [1]Department of Biology, Rhodes College, Memphis, TN. [2]Department of Botany, University of Georgia, Athens, GA.

The hyphal wall is the cell structure most responsible for mediating the varied interactions between the fungal cell and its environment. It is a dynamic organelle, assembled *in situ* from exported precursors and subject to

developmentally regulated modulation during such processes as branching and sporulation. The architectural relationships between the numerous polysaccharides and glycoproteins of the wall are little known, as are the steps by which the complex fabric of the wall is assembled and modified. In an effort to isolate mutants deficient in any of a wide range of cell wall assembly steps, we have identified 52 strains from an *Aspergillus nidulans* mutant collection, which show hypersensitivity to the chitin synthase inhibitor Calcofluor White (Blankophor BBH) at concentrations having little effect on wildtype strains. The phenotype appears as reduced hyphal growth rate, blocked or delayed conidium germination, or abnormalities of hyphal form. The screening strategy is based upon the premise that mutants with already-weakened walls will be less able to endure an additional disturbance from a sublethal concentration of the inhibitor. To date, ten single-gene mutations have been identified, and one (designated *cal*A) has been shown to be closely linked to the *arg*B locus (chromosome III). Assignment of the remaining nine mutations to linkage groups is underway, and all are being assigned to complementation groups.

27 **A PAK-like protein kinase is required for maturation of young hyphae and septation in the filamentous ascomycete *Ashbya gossypii*.** Yasmina Ayad-Durieux, Philipp Knechtle, Fred Dietrich and Peter Philippsen. Biozentrum, University of Basel, Applied Microbiology, Basel, Switzerland

Filamentous fungi grow by hyphal extension, which is an extreme example of polarized growth. In contrast to yeast species, where polarized growth of the tip of an emerging bud is temporally limited, filamentous fungi exhibit constitutive polarized growth of the hyphal tip. In many fungi including *Ashbya gossypii*polarized growth is reinforced by a process called hyphal maturation. Hyphal maturation refers to the developmental switch from slow-growing hyphae of young mycelium to fast-growing hyphae of mature mycelium. This process is essential for efficient expansion of mycelium. We report for the first time on the identification and characterization of a fungal gene important for hyphal maturation. This novel *A. gossypii*gene encodes a presumptive PAK-like (p21-activated kinase) kinase. Its closest homolog is the *S.cerevisiae*Cla4 protein kinase, the *A. gossypii*protein is therefore called AgCla4p. *Agcla4*deletion strains are no longer able to perform the developmental switch from young to mature hyphae, and GFP (green fluorescent protein)-tagged AgCla4p localizes with much higher frequency in mature hyphal tips than in young hyphal tips. Both results support the importance of AgCla4p in hyphal maturation. AgCla4p is also required for septation, indicated by the inability of *Agcla4*deletion strains to properly form actin rings and chitin rings. Despite the requirement of AgCla4p for the development of fast-growing hyphae, AgCla4p is not necessary for actin polarization per se, because tips enriched in cortical patches and hyphae with a fully developped network of actin cables can be seen in *Agcla4*deletion strains. The possibility that AgCla4p may be involved in regulatory mechanisms that control the dynamics of the actin patches and/or actin cables is discussed.

28 **Molecular analysis of the *Aspergillus nidulans* morphogenesis locus, *hypA*.** Susan Kaminskyj[1], Xianzong Shi[1], John Hamer[2]. [1]Biology Dept. U Saskatchewan, SK Canada, and [1]Paradigm Genetics, Research Triangle Park NC, USA.

Aspergillus nidulans hypA1 strains are wildtype at 28 C but at 42 C grow slowly with wide hyphae, frequent branches and septa, and delayed sporulation. *hypA* was cloned by complementation, encoding a predicted 1418 amino acid peptide with a molecular weight of 155.9 kDa and a pI of 5.77. Motif searching suggests that the *hypA* product is cytoplasmic and may be regulated by phosphorylation via cAMP- and/or Ca-dependent kinases. *A. nidulans hypA* has closely related sequences in *Neurospora crassa* and *Schizosaccharomyces pombe* whose function is unknown, and *Saccharomyces cerevisiae TRS120*, which is a putative regulatory subunit in the TRAPP complex that mediates ER to cis-Golgi transport. The *N. crassa* and *S. pombe* genes repaired the *hypA1* phenotype by homeologous recombination but did so in different ways; repair of *hypA1* by *TRS120* was unsuccessful. Repair by the *S. pombe* sequence created a new *hypA* allele. We deduced six conserved blocks amongst these four sequences and showed that *hypA1* is due to a point mutation causing a non-conservative amino acid change in block A. Unlike *TRS120*, which is essential in *S. cerevisiae*, *hypA* knockout strains grow slowly, but only on 1M sucrose agar at 28 C, and produce sparse but viable spores after three weeks. Shifting *hypA* knockout strains to 42 C was lethal. *hypA* has roles in hyphal growth and morphogenesis that may be mediated through regulated secretion.

29 **Differential expression of two novel *Aspergillus fumigatus* putative drug efflux genes in mutant strains with high level resistance to itraconazole.** Adriana M. Nascimento[1], Steven Park, S.A.E. Marras, R. Kashiwazaki, Gustavo H. Goldman[1], and David. S. Perlin. Public Health Research Institute, USA and [1]FCFRP - Universidade de Sao Paulo, Brazil

Aspergillus fumigatus is an opportunistic pathogen that can cause life-threatening invasive disease in severely immunocompromised patients. For such patients, the treatment options are limited to therapy with amphotericin B and/or itraconazole. As a preliminary step to investigate the underlying resistance mechanisms in this clinically important pathogen, *A. fumigatus* mutants resistant to itraconazole (RIT) were selected *in vitro* following UV irradiation. A total of fifty-five strains were isolated that grew in presence of up to 100 mg/ml of itraconazole. To evaluate whether ATP Binding Cassete (ABC) multiple drug resistance (MDR) pumps contributed to the observed drug resistance phenotype of the RIT mutants, PCR amplification using degenerate primers to a conserved region within the ABC superfamily was used to identify genes encoding potential MDR efflux pumps. The PCR fragments obtained were cloned and sequenced, and six of these clones corresponded to potential MDR type genes. Two of these novel *A. fumigatus* putative drug efflux genes, designated *mdr*3 and *mdr*4, showed differential, drug-induced, gene expression and were evaluated in more detail. Real-time assays with molecular beacon probes were used to quantitatively measure mRNA levels in the 23 mutant strains grown in the presence and absence of itraconazole (10 or 100 ug/ml). Two RIT strains, RIT11 and RIT13, were identified that showed up to 200-fold increased levels of *mdr*3 and *mdr*4 mRNA after treatment with itraconazole. Our results suggest that these novel drug efflux genes may contribute mechanistically to the itraconazole resistance phenotypes displayed for the *A. fumigatus* RIT mutant strains, and may have clinical significance.

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30 **Molecular responses in *Aspergillus nidulans* to treatment with *Streptomyces*-produced antibiotics.** Petter Melin[1], Johan Schnurer[1] & Gerhart Wagner[2]. [1]Department of Microbiology, Swedish University of Agricultural Sciences [2]Institute of Cell and Molecular Biology, Uppsala University

In the search for fungal-antagonistic bacteria we previously identified a strain of *Streptomyces halstedii* that produces the macrolide-antibiotics bafilomycin B1 and C1. Bafilomycins are known to inhibit vacuolar ATPases. Filamentous fungi exposed to these antibiotics characteristically display reduced growth rate and increased branching frequency. We previously developed an experimental model to study the molecular response in fungi exposed to bafilomycin B1. By using mRNA differential display, we have identified five genes in *Aspergillus nidulans* whose expression changes upon bafilomycin treatment. We are now proceeding to test for the biological function of the detected genes, starting with binB, whose expression had been shown to be up- regulated approximately 30 times. We have also initiated a proteome analysis to monitor expression changes at the protein level. We expect that the complementary results from RNA (differential display) and proteome analysis will be useful to identify more putative targets of the antibiotics, and also will provide an estimate of the sensitivity of the two screening methods. In the proteomics study we used the antibiotic concanamycin A, which induces phenotypic changes in the fungus that are virtually identical to those observed with Bafilomycin. Among the 130 most prominent proteins spots analyzed by 2D gel electrophoresis, 23 proteins show a change in abundance of at least two-fold. The identity of two proteins with decreased abundance was identified as CPCB (involved in regulation of sexual development) and GPD (a central protein in glycolysis), respectively. We cannot, at this point, provide a biological explanation for the reduced abundance of these protein as a function of antibiotics treatment. The remaining proteins are still under investigation.

31 **The MAP kinase cascade and cAMP signaling modulate pH-induced yeast to mycelium change in *Ustilago maydis.*** Alfredo D. Martinez-Espinoza [1,2], Jos Ruiz-Herrera[2], Claudia G. Leon-Ramirez and Scott E. Gold[1]. [1]Plant Pathology Dept. University of Georgia, Athens GA, 30602-7274, USA. [2]Departamento de Ingenieria Genetica, Centro de Investigacion y de Estudios Avanzados del Instituto Politecnico Nacional. Unidade Irapuato, Apartado Postal 629, 36500 Irapuato, Gto. Mexico.

*Ustilago maydis*is a worldwide smut pathogen of maize. In its saprophytic phase, the fungus is haploid and grows as a budding yeast. A pathogenic dikaryotic mycelium is produced after mating of compatible sporidia. Alternatively to mating, acidic pH of the media can induce the dimorphic yeast- mycelium switch of this fungus. A mutational analysis of two important signal transduction pathways showed how the pH mycelial induction might be operating. Mycelial formation *in vitro*was inhibited by the addition of exogenous cAMP in all growth conditions. cAMP levels drop dramatically and were maintained at low levels when the fungus was grown at pH 3.0. Mutants affected in the regulatory subunit of PKA still showed a mycelial phenotype at low pH, indicating an alternative effector of the cAMP signal. With respect to the MAP kinase pathway, it was observed that mutations in any of the members:

MAPK, MAPKK, MAPKKK or a putative adaptor protein, lost their capacity to form mycelium in vitro. Interestingly, a mutation on the transcriptional regulator *prf1*, was still capable of forming mycelium under acidic conditions. These results suggest that the normal condition of growth of *U. maydis*is budding, which is maintained at least in part, by a mechanism of repression exerted through PKA signaling. When levels of cAMP descend, the fungus exhibits a filamentous morphology using a mechanism that requires the MAPK pathway. This pathway can be stimulated under natural conditions in the dikaryon by a b-dependent mechanism or in vitro under low pH conditions.

32 **Expression of green fluorescent protein in *Neurospora crassa***. Michael Freitag, Lynda Ciuffetti* and Eric U. Selker. Institute of Molecular Biology, University of Oregon, Eugene, and *Department of Botany and Plant Pathology, Oregon State University, Corvallis

Since the first report on heterologous expression of GFP in *Escherichia coli* and *Caenorhabditis elegans*, GFP has become an amazingly versatile and useful protein marker in many experimental systems. GFP and GFP fusion proteins have been successfully expressed under the control of both endogenous and heterologous promoters in some fungi, but not previously in *N. crassa*. After transformation of *N. crassa* with pCT74, a plasmid that carries a modified form of the GFP gene under the control of the *Pyrenophora tritici-repentis ToxA* promoter and the *E. coli hph* gene as a selectable marker, we detected high GFP levels in the cytoplasm and nuclei of both hyphae and macroconidia. GFP appeared excluded from vacuoles and mitochondria. Transformants exhibited variable GFP expression levels and patterns, presumably because of differences in their copy number and site of integration. We therefore targeted a single copy of GFP to the *his-3* locus. The resulting transformants showed uniform and high-level expression of GFP. To assess the sexual stability of these transformants and expression of GFP in sexual tissues, we crossed single copy GFP transformants with wildtype strains; GFP was easily detected in developing ascospores, proving GFP to be a useful ascospore color marker. One explanation for previous difficulties in creating successfully expressing Neurospora-GFP fusion proteins may be the relative strength of the available promoters. We are presently testing this and are performing a promoter trap screen to identify useful Neurospora promoters. Low levels of GFP expression may also be explained by gene silencing, for example by DNA methylation. This possibility is also being examined.

33 **Characterization and cloning of the *Neurospora crassa* DNA repair gene, *upr-1*.** Wataru Sakai, Chizu Ishii and Hirokazu Inoue. Saitama University, Urawa, Japan

Two mutagen-sensitive mutants, *upr-1* and *mus-26*, share the same phenotype; (1) sensitive to UV light and 4-NQO and only slightly sensitive to MMS and gamma rays, (2) low reversion frequency and (3) partial defective in photoreactivation. Genetic analysis showed that they belong to the same repair pathway. However, there is no other mutant that shows epistasis to *upr-1* and *mus-26*. Therefore the function of these products is unknown. The *upr-1* gene locates at the left arm of linkage group I, near mating type (*mt*). In order to isolate the *upr-1* gene, we did chromosome walking from the *mt* locus to the right direction and contiguous clones covered about 300-400 kb genomic region. Some clones complemented the temperature- sensitive mutant *un-16*, which is mapped between the *mt* locus and the *upr-1* locus. Based on the obtained *un-16* gene sequence, the MIPS *Neurospora crassa* database was screened for contiguous sequence. We found that the homolog of budding yeast *REV3* is next to the *un-16* gene. Phenotype of the yeast *rev3* mutant is similar to that of the *upr-1* mutant. We are now testing whether the *upr-1* gene is identical to the *REV3* homolog, and characterizing RIP mutants of the *N. crassa REV3* homolog.

34 **Characterization of mutations in the two-component histidine kinase gene of dicarboximide-resistant field strains of *Botrytis cinerea*.** Michiyo Oshima[1], Makoto Fujimura[1], Noriyuki Ochiai[1], Akihiko Ichiishi[1], Takayuki Motoyama[2], and Isamu Yamaguchi[2]. [1]University of Toyo, Dept of Life Science, Itakura, Gunma, Japan. [2]Microbial Toxicology Lab., RIKEN Institute, Wako, Saitama, Japan

The mutations in putative osomosensor histidine kinase gene (*os-1*) in *Neurospora crassa* confer not only osmotic sensitivity but also resistance both to dicarboximides and phenylpyrroles. In a plant pathogenic *Botrytis cinerea*, the dicarboximide-resistant strains isolated from practical fields are still sensitive to phenylpyrroles without osmotic sensitive phenotype, although most dicarboximide-resistant mutants isolated under laboratory condition exhibit both osmotic sensitivity and resistance to phenylpyrroles. To investigate the possibility that mutations of histidine kinase gene (*BsOS1*) confer dicarboximide-resistance in field strains of *B. cinerea*, we cloned *BsOS1* genes from both

dicarboximide-sensitive and resistant field strains by PCR amplification and compared their sequences. The overall domain organization was conserved between the os-1p and BcOS1p including the N-terminal domain that contains tandem repeats of 90 amino acids. All dicarboximide-resistant field strains of *B. cinerea* were found to contain a single base pair mutation in their osmosensor histidine kinase gene that resulted in an amino acid substitution in second unit of 90 amino-acids tandem repeats of BcOS1p. In four resistant isolates, coden-76 of second amino-acid repeat, which encodes isoleucine in sensitive strains, was converted to a coden for serine. The coden-70 of second repeat for leucine was converted to a coden for proline in one resistant strain. These results strongly suggests that the amino acid substitutions within second repeat of BcOS1p are responsible for phenotypes of field resistant isolates (resistant to dicarboximides, sensitive to phenylpyrroles, and insensitive to osmotic stress) in *B. cinerea*.

35 **Characterization of he kex2 endopeptidase genes from *Aspergillus oryzae* and *Aspergillus nidulans*.**
Y.Yamgata[1], M. Yamamoto[1], A. Nojima[1], Y. Katsuno[1], K. Abe[1], T. Nakajima[1], T. Akao[2], O. Akita[2], M. Sano[3], M. Machida[3]. [1]Tohoku University Sendai, Japan. [2]National Resarch Institute of Brewing, Saijo, Japan. [3]National Institute of Bioscience and Human-Technology, Tskuba, Japan

The details of fungal protein expression system have been not clear, and it has been thought as same as the yeast secretion system. As the first step to clarify the protein expression system of *Aspergillus*, we cloned and sequenced genes for processing protease of *Aspergillus nidulans* and *A. oryzae*. As a result of BLAST search against EST database of *A. nidulans*, we found 600-bp sequence homologous to yeast kex2 gene, and the sequence was amplified with PCR. Complementary DNA sequence was obtained by screening a lambda-ZAP cDNA library of *A. nidulans* A69 strain with the amplified fragment. Obtained gene contained ORF of 2460 bp, and it encoded 819 amino acid residues. It was 40% identical with yeast kex2 protease. Then as we searched for homologous sequences with the *A. nidulans* kex2 homologue in *A. oryzae* EST database, the sequence of 70 % identical with kex2 homologue was found. The sequence contained ORF of 2511 bp, and it encoded 836 amino acid residues. These two enzymes and *A. niger* kex2 protein were 70 % identical with each other, and 40 % identical with yeast kex2 protease. The amino acid residues of catalytic triad with serine protease, the amino acid sequences of transmenbrane domain and p-domain were well conserved. The gene from *A. oryzae* was introduced into *E. coli* with pET expression vector, the hydrolytic activities against synthetic peptides were observed in *E. coli* lysate. The facts above indicated that these two genes encoded kex2 maturating protease of *A. nidulans* and *A. oryzae*.

36 **Molecular cloning and characterization of a novel two-component signaling histidine kinase gene *tcsB* (*NHK1*) of *Aspergillus nidulans*.** Yasuaki Katsuno, Kentaro Furukawa, Keietsu Abe*, and Tasuku Nakajima. Department of Molecular and Cell Biology, Graduate School of Agricultural Science, Tohoku University, Sendai, JAPAN

We cloned and characterized a novel *Aspergillus nidulans* histidine kinase gene, *tcsB* (*NHK1*) encoding a two-component signaling protein homologous to the yeast osmosensor synthetic lethal of N-end rule 1 (SLN1) which transmits signals through the high-osmolarity glycerol response 1 (HOG1) mitogen- activated knase (MAPK) cascade in yeast responding to environmental osmotic stimuli. In the *A. nidulans* EST database (http://www.genome.ou.edu/ fungal.html), we found three EST sequences homologous to histidine kinase conserved regions, H-boxes, N-boxes, and regulator domains respectively observed in yeast *SLN1* or *Neurospora crassa NIK-1*. Thus, DNA fragments containing either of the three EST sequences were amplified by PCR and used for screening of an *A. nidulans* cDNA library as probes. From the screening, we isolated a positive clone containing a 3,210 bp long open reading frame that gave a putative protein consisted of 1,070 amino acids, and designated the gene *tcsB* . The predicted *tcsB* protein has two putative transmembrane regions in the N-terminal half and has structural similarity to the yeast Sln1p. Overexpression of the *tcsB* cDNA suppressed the lethality of the temperature-sensitive osmosensing-defective yeast mutant *sln1-ts*. By contrast, *tcsB* cDNAs in which conserved histidine or aspartate residues had been substituted (H552Q, D989N) failed to complement the *sln1-ts* mutation, indicating that the *tcsB* protein functions as a histidine kinase.

37 **Nuclear control of mtDNA maintenance: identification and characterization of two orphan genes in *Podospora anserina*.** M. Dequard-Chablat, V. Contamine and M. Picard. Institut de Genetique et Microbiologie, CNRS-UMR 8621 Universit Paris-Sud, Bat 400 91405-ORSAY cedex FRANCE

In *P. anserina*, a degenerative process (premature death) is linked to the accumulation of mtDNA molecules (delta1) carrying a large and specific deletion. It occurs only in the presence of some mutations of the *AS1* gene encoding a cytosolic ribosomal protein. Our working hypothesis is that these mutations alter translation, notably for mRNAs encoding proteins required for the maintenance/distribution of the mitochondrial genome. Search for mutations which either delay or suppress the accumulation of delta1 should help to identify the relevant genes. Unexpectidly, the first two genes identified by this procedure are probably not the targets of the *AS1* effect: mutations in these genes might indirectly counteract this effect through modifications in the process for protein import into mitochondria. In contrast, the *SMP1* gene is a good candidate for an *AS1* target. It was identified as a gene which, in two copies, delays premature death. This is an orphan gene which probably encodes a HMG protein located into mitochondria. The *rmp1* gene is also an orphan gene. It was discovered as a gene exhibiting natural allelic forms which belong to two classes: class 1 alleles lead to the premature death syndrome *per se* while class 2 alleles delay accumulation of delta1. Sequencing of six class 1 and two class 2 alleles has shown that the second forms encode a truncated protein. Experiments are in progress to understand the role(s) of these two genes in mtDNA maintenance and distribution.

38 **Peroxisomal ABC transporters, inter-organelle cross-talk and development in *Podospora anserina*.**
Stéphanie Boisnard, Gwenaël Ruprich-Robert, Véronique Berteaux-Lecellier, Denise Zickler and Marguerite Picard. Institut de Génétique et Microbiologie, UMR 8621, Universit Paris-Sud, Bat. 400, 91405 Orsay Cedex, France

Although recent advances have greatly expanded our knowledge of peroxisome biogenesis, the connection between peroxisomal deficiencies and developmental defects remains largely unknown. We have previously shown that *pex2* mutants of the filamentous fungus *Podospora anserina*, impaired in peroxisome assembly, are also defective in sexual differentiation: dikaryotic mutant cells remain in a proliferative stage and are unable to differentiate into meiocyte and enter meiosis. To confirm the involvement of peroxisomes in sexual differentiation, we took advantage of the unexpected link found in mammals between PEX2 (general loss of peroxisomal function; Zellweger syndrome) and peroxysomal ABC transporters: overexpression of either ALDP (Adrenoleukodystrophy) or PMP70 (whose role(s) remain unclear) suppresses defects in peroxisomal assembly in cells that are deficient for PEX2. We show that the human PMP70 protein can restore sexual differentiation and peroxisome biogenesis in a *Podospora* peroxisome-deficient *pex2* mutant. These observations confirm that the developmental defect observed in the mutant is a consequence of a peroxisomal dysfunctionment and rule out a role of PEX2 *per se*. Moreover, we show that expression of the human *PMP70* cDNA in a *Podospora* wild-type strain causes developmental defects. To precise the peroxisomal requirements during sexual development, we also performed a search for extragenic suppressors of *pex2* mutants. This led to the characterization of six complementation groups among which the mitochondrial citrate synthase gene (*cit1*) has been identified. *cit1* mutations act as partial suppressors of *pex2* mutants (*via* a process which probably involves an inter-organelle cross-talk) and disclose a meiotic checkpoint at the diffuse stage.

39 **Apical growth and mitosis do not compete for the same microtubule resources in *Aspergillus nidulans.*** M. Riquelme[1], R. Fischer[2] and S. Bartnicki-Garcia[1]. [1]Department of Plant Pathology. University of California, Riverside. Riverside, CA 92521. [2] Max- Planck-Institut f r Terrestrische Mikrobiologie. Marburg, Germany.

We conducted a simultaneous analysis of nuclear division and apical growth in living fungal hyphae by fluorescence and phase-contrast microscopy, respectively, to determine if the key parameters of apical growth (elongation rate and Spitzenkörper behavior) are affected during mitosis. We used *Aspergillus nidulans* strain SRS27, in which nuclei are stained with GFP (Suelmann *et al*., 1997). During mitosis, nuclei became invisible as the GFP was released from the nuclei and the entire hypha became diffusely fluorescent. Interphase nuclei and their disappearance during mitosis could also be observed by phase-contrast microscopy but with considerable difficulty. Contrary to earlier predictions, our analysis showed that there is no disruption of apical growth during mitosis. There was no decrease in the rate of hyphal elongation or any alteration in Spitzenkörper behavior before, during or after mitosis. The distribution of cytoplasmic microtubules (MTs) in young hyphae of *A. nidulans* was examined by immunofluorescence to get a better understanding of the status of the microtubular cytoskeleton during mitosis. In addition to the typical mitotic spindles, cytoplasmic MTs were clearly evident in cells arrested at metaphase. Our findings disprove the common belief that apical growth and mitosis compete for the same pool of MTs. Presumably, the population of cytoplasmic MTs involved in apical growth operates independently of that involved in mitosis.

40 **Comparative morphometric analysis of lateral and apical branching in hyphae of *Neurospora crassa***. M. Riquelme[1], G. Gierz[2], and S. Bartnicki-Garcia[1]. Departments of [1]Plant Pathology and [2] Mathematics. University of California, Riverside. Riverside, CA 92521.

We have used high-resolution video-enhanced light microscopy (VELM) to measure hyphal growth kinetics, with high accuracy, and to examine, in fine detail, the intracellular events that lead to lateral and apical branching in hyphae of *Neurospora crassa*. We found remarkable differences in the events preceding lateral vs. apical branching. Lateral branches emerged without affecting the growth of the main hypha. Lateral branch formation did not interfere with either the elongation rate of the primary hypha or the behavior of its Spitzenk÷÷rper (Spk). During emergence of a lateral branch, a new Spk was formed (at 15-30 m behind the apex) without affecting the behavior of the primary Spk. In sharp contrast, apical branching was preceded by a series of intracellular events at the parental hyphal apex. The sequence involved: 1) Cytoplasmic contraction, followed by 2) retraction/dislocation, and disappearance of the Spk. During this period, hyphal elongation decreased sharply accompanied by a transient phase of non-polar isotropic growth. About 2 min after the Spk retraction, active growth resumed with the formation of two or more apical branches, each one with a Spk formed de novo by gradual condensation of phase-dark material (vesicles) around an invisible nucleation site.

41 **Regulation of cytokinesis in *Ustilago maydis.*** Helge Hudel, Britta Abel, Marisa Piscator, Michael Bölker. University of Marburg, Department of Biology, Marburg, Germany

The phytopathogenic fungus *Ustilago maydis* exhibits a dimorphic life style. Haploid sporidia grow yeast-like by budding and are non-pathogenic. By UV mutagenesis we have identified two genes that are involved in the regulation of morphogenesis and dimorphism. *don1* and *don3* mutant cells fail to separate after nuclear division and septum formation. The *don1* gene encodes a guanine exchange factor (GEF) specific for members of the Rho/Rac/Cdc42 family, the *don3* gene codes for a member of the STE20 like kinases that are known to be activated by Rho/Rac proteins. The Ras-like GTPase Cdc42 is supposed to be involved in this regulatory cascade. By using an in vitro kinase assay we could demonstrate that Cdc42 is able to activate the Don3 protein kinase. To characterize the role of Cdc42 in more detail we are currently testing specific mutants of Cdc42 for their effect on cytokinesis and cell separation. We propose that Don1 and Don3 control via Cdc42 the growth of a secondary septum which is required to form a fragmentation zone.

42 **Pheromone discrimination of chimeric *Balpha* receptors in *Schizophyllum commune***. Susanne Gola, and Erika Kothe. Dept. Microbiology, Friedrich-Schiller-University, Jena, Germany.

The *Balpha* mating type locus of the basidiomycete *Schizophyllum commune* contains a pheromone receptor system which is capable of ligand discrimination to confer mating specificity to a haploid strain. Each pheromone receptor induces *B*-dependent development upon activation by pheromones of eight non-self specificities, while self-pheromones are not bound. In order to define specificity domains of receptors, chimeric receptors were constructed using the receptor genes *bar1* of *Balpha1* specificity and *bar2* of *Balpha2* specificity. Chimeras were transformed into a *Bnull* strain lacking endogenous pheromone and receptor genes. Specificities of the chimeric receptors were analysed by mating to tester strains of all nine *Balpha* specificities. Matings revealed four phenotypes of the chimeras: Two of the receptors carried *Balpha1* specificity. One showed an altered, highly discriminative exclusion profile. The remaining three receptors were promiscuous, one of them was also constitutive. The group of promiscuous receptors showed activation by pheromones of all nine *Balpha* specificities, i.e. no discrimination between self and non-self mates. To distinguish activation profiles of chimeric receptors single pheromone genes were expressed under control of the strong *tef1* promotor in the *Bnull* recipient strain and then mated to transformants expressing chimeric receptors. Distinct pheromone-receptor pairs detected differential responses of the promiscuous receptors to specific pheromones. Thus, the receptors are not accepting any pheromone as expected for truly promiscuous phenotypes. They still show differential activation profiles, albeit with a new assortment of pheromones. In addition, the profiles of activation vs. rejection differed between the promiscuous receptors analyzed.

43 **A linear plasmid from *Blumeria graminis* f. sp. *hordei*.** Henriette Giese[1], Bjarne Stummann[2] and Solveig Christiansen[3]. [1]Royal Agricultural and Veterinary Universitya, Ecology, Copenhagen, Denmark. [2]Vet. Agri. University , Ecology, Copenhagen Denmark. [3]Risoe National Laboratory, Plant Biology, Roskilde, Denmark.

A 8.0 kb linear plasmid from the obligate biotrophic fungus *Blumeria graminis* f.sp. *hordei* (synonym *Erysiphe graminis*) (Bgh), causing the disease powdery mildew on barley, was characterised by sequence analysis. The plasmid contains two identical terminal inverted repeats (TIR) of 610 bp. Two open reading frames on opposite strands and with start codons 570 bp from the plasmid ends were identified, one encoding a DNA polymerase with 1030 aa and the other an RNA polymerase with 961 aa. These genes are transcribed throughout fungal development. A putative 11 bp ARS consensus sequence ATTTATATTTA was identified within the TIR elements. Sexual transmission of the plasmid was studied in a cross between two Bgh isolates characterised by presence and absence of the plasmid and with different mitochondrial genotypes. The plasmid was transferred to 75% of the 87 progeny isolates and 66% of the isolates had the mitochondrial genotype of the isolate without the plasmid. The mitochondrial genotype originally harbouring the plasmid may have a dependence on the plasmid as only 4% of the isolates had this genotype without the plasmid. No transfer of the plasmid was observed between two Bgh isolates co-cultivated for over a year on a common host variety.

44 **Isolation and characterization of sexual sporulation mutants of *Aspergillus nidulans*.** K. Swart, D. van Heemst, M. Slakhorst, F. Debets and C. Heyting. Laboratory of Genetics, Department of Plant Sciences, Wageningen University, 6703 HA Wageningen, The Netherlands.

For the genetic dissection of sexual sporulation in *Aspergillus nidulans*, we started a collection of ascosporeless mutants. After mutagenization of conidiospores with high doses of UV, we isolated 20 mutants with defects in ascospore formation. We crossed these mutants in two successive rounds with the wildtype strain. Eighteen of the 20 isolated mutants produced progeny with the original mutant phenotype in these crosses, and these mutants were further analyzed. All 18 analyzed mutations were recessive to wildtype. We assigned them to 15 complementation groups, based on crosses between mutants. The mutants could be classified as follows according to their cytological phenotype: (1) no croziers; (2) arrest at pre-karyogamy; (3) arrest in early meiotic prophase; (4) arrest in late meiotic prophase; (5) arrest in meiotic metaphase I; (6) defective post meitotic mitosis and/or deliniation of ascospores; and (7) slow progression through the post meiotic stages of ascospore formation. A large proportion of the mutants, namely 11 out of 18, arrested in meiotic prophase or metaphase I. We discuss a possible approach for isolating the wildtype aleles of the genes that carry the sexual sporulation mutations.

45 **Fungal cell death.** Charlotte Thrane and Stefan Olsson. Ecology, Royal and Veterinary University Coppenhagen, Denmark

In plants, controlled cell death with similarities to animal apoptosis is thought to occur and biochemical signs like caspase 3-like activities and PARP- cleavage have been observed during patogen infection or after toxin treatment. In fungi, it is still speculative but parts of the protein machinery (Bcl-2 and Bax) were identified by chemical treatment of *Mucor racemosus*. Some form of controlled cell death has also been suggested in cord formation, heterokaryon incompatibility in Neurospora crassa, and in the development of primordia in *Agaricus bisporus*. We have studied fungal cell death in two different systems; toxin (or antimicrobial) induced and developmental. Our data indicate that proteins central in animal apoptosis are present in fungi. However, there are several large differences in the basic cellular organisation between fungi and animal cells that might signify differences in a programmed cell death pathway. The fungal cell wall is one such difference which is significant in toxin or antimicrobial induced stress: The fungal cell wall can function to protect the fungus from extracellular stress by sealing of the boundary to the environment, and the wall is, further, a site for accumulation of antimicrobial compunds. This suggests that the signs of fungal cell death as a response to stress differ from wall-less animals and more resemble stress responses in plants. On the other hand, developmental cell death in fungi and animals might be more similar.

46 **Stress induced responses in *Fusarium culmorum*.** Henriette Giese, Stefan Olsson, Jakob Skov, Carsten Tobiasen, Morten Grell, and Bjarne Stummann. Ecology, Royal and Veterinary University Coppenhagen, Denmark

Fusarium culmorum is the most frequent Fusarium species in Danish soils and is believed to be the major cause for Fusarium head blight of barley in Denmark. Fusarium species are also used as production organisms by the biotechnological industry and it is important to gain insight into the processes that lead to mycotoxin production. The aim of our research is to characterize the processes leading to toxin production in *Fusarium culmorum* when the fungus is subjected to different stress conditions. Defined culture systems for *F. culmorum* have been developed.

Stress applications such as nutrient and oxygen starvation, heat, pH variation and chemical compounds are tested. The entire mycelium is stressed in a liquid culture system to get a definite starting point to facilitate molecular analyses of fungal stress reactions as a series of events triggered by a specific external factor. The fungal stress responses are characterised by microscopy to monitor cytological changes that can be used to verify reproducible stress treatments. 2-D electrophoresis of proteins extracted from fungal cultures subjected to different types of stress is carried out to detect the resulting change and develop a databank of protein patterns derived from fungal cultures of different age and stress treatments. A long term perspective is to analyse individual protein spots by mass spectrometry to identify the proteins that are characteristic for specific culture conditions. A transformation system for the fungus is under development and initially a vector containing a constitutive promoter in front of a Ca2+ responsive reporter gene will be used. This will permit direct assessment of stress induction in Fusarium culmorum. Promoters from genes that are induced by specific treatments will at later stages be used in transformation experiments to study gene expression in stressed fungal cultures.

47 **Characterization of *nop-1* expression during development in *Neurospora crassa.*** Jennifer A. Bieszke, Donald O. Natvig, and Katherine A. Borkovich. University of New Mexico, and the University of Texas-Houston Medical School.

Opsins are seven-transmembrane helical apoproteins that form light absorbing pigments upon binding retinal. Genes encoding opsins were found exclusively in animals and the archaea until the discovery of *nop-1* in the filamentous fungus *Neurospora crassa.* Previously, we have shown that heterologously expressed NOP-1 in *P. pastoris* membranes could bind all-*trans* retinal ($lambda_{max}$ = 534nm), and undergo a photochemical reaction cycle similar to archaeal rhodopsins. Also, we found that *nop-1* is expressed to high levels under conditions that favor conidiation. Further analysis of *nop-1* mRNA levels during conidial development demonstrates that the *nop-1* transcript first appears early in conidiation (4 hrs) and persists throughout conidiophore development and conidial maturation (16 hrs). Since several genes specific to conidiation are dependent on blue-light, *nop- 1* expression was evaluated in the *wc-1* and *wc-2* strains. Preliminary evidence suggests that *nop-1* expression is independent of blue-light control. Finally, a NOP-1 antibody has been generated and the expression and localization of NOP-1 throughout *N. crassa* development is currently being investigated.

48 **Cloning of the Neurospora uvs-3 gene.** Schroeder, Alice L[1],Kazama, Yusuke[2], Ishii, Chizu [2] and Inoue, Hirokazu [2]. [1]School of Molecular BioSciences, Washington State Univ.,USA, [2] Lab. of Genetics, Saitama Univ., JAPAN

A genomic DNA fragment which complemented the methyl methanesulfonate (MMS) sensitivity of the DNA repair mutant, *uvs-3*, was cloned. The 4.5 Kb XhoI-BglII fragment of the cosmid X:18E9 contains two long ORFs divided by a putative intron. The deduced protein is 883 a.a. long with no detectable termination codon, and shows about 35 % similarity and 25% identity to the Aspergillus nidulans uvsD gene product. The *uvsD* mutant of Aspergillus is thought to be involved in checkpoint control and shares many characteristics with the *uvs-3* mutant, including a broad mutagen specificity and retention of photoreactivation ability for at least 4 hours after UV irradiation. The introduction of *uvsD* cDNA partially restored MMS and UV resistance to the *uvs-3* mutant. This suggests that *uvs-3* plays a role in checkpoint control in Neurospora. Putative null mutants created by ripping shared a similar phenotype with the original *uvs-3* mutant. Full resistance to MMS was restored in the ripped mutant by introduction of the whole cosmid fragment, while the original mutant gained only partial resistance with the cosmid. This result indicates that original mutation in the *uvs-3* mutant may be a semi- dominant. Northern blot analysis of the *uvs-3* gene indicated that the *uvs-3* gene was constitutively expressed at a low level. The expression was reduced after UV irradiation but recovered within 60 min. By 90 to 120 min after irradiation it had increased to a level several times higher than the constitutive level. This work was supported in part by a NSF US - Japan Cooperative Research Grant.

49 **Protein kinase a control in glucose and nitrogen sensing in *Saccharomyces cerevisiae*.** Inge Holsbeeks, Monica C.V. Donaton, Ole Lagatie, Marion Crauwels, Joris Winderickx, Johan M. Thevelin. Laboratory of Molecular Cell Biology, K.U. Leuven, Kardinaal Mercierlaan 92, B-3001 Leuven, Flanders, Belgium.

Protein kinase A (PKA) and the PKA-related pathways play a major role in nutrient induced signal transduction in Saccharomyces cerevisiae. These PKA-pathways are important for control of a variety of growth related metabolic

properties such as regulation of trehalose and glycogen content, expression of STRE-controlled genes and ribosomal gene expression (1). Addition of glucose to cells deprived for glucose triggers a cAMP-increase which is dependent on the Gpr1-Gpa2 G-protein coupled receptor system. This cAMP-signal triggers a PKA-mediated protein phosphorylation cascade that affects the different targets of the cAMP-PKA-pathway. When cells starved for an essential nutrient in presence of glucose are replenished with the essential nutrient, a similar PKA-mediated protein phosphorylation cascade occurs, but without cAMP-increase. Because of requirement of glucose and complete growth medium this pathway is called "fermentable growth medium induced" (FGM) pathway (2). This pathway has been studied in nitrogen starved cells on a glucose medium to which a nitrogen source was added again. Activation of the FGM-pathway is largely dependent on the general amino acid permease Gap1. In a gap1 strain some amino acids can still be transported into the cell but they can't activate the PKA-targets. Furthermore some C-terminal truncations of Gap1, which have no effect on the transport capacity of Gap1, resulted in reduced trehalase activation after addition of nitrogen. On the other hand these truncated alleles of Gap1 showed a constitutive activation of all other PKA-targets measured irrespective of the presence of the nitrogen source. These results provide strong evidence that Gap1 acts as a sensor for amino acid activation of the FGM-pathway.

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50 ***het-c* vegetative incompatibility causes well-timed mycelial growth arrest and hyphal compartmentation and death in *Neurospora crassa.*** Qijun Xiang, Magdalen Barton, Gopal Iyer, and N. Louise Glass. University of California, Plant and Microbial Biology, Berkeley, CA,

In filamentous fungi, the formation of stable heterokaryons between different strains is genetically controlled by *het* loci. The *het-c* locus is one of eleven *het* loci in *Neurospora crassa*. Vegetative incompatibility mediated by differences at *het-c* has at least three phenotypic aspects: mycelial growth arrest, suppression of conidiation, and hyphal compartmentation and death (HCD). Hyphal compartmentation death rates are relatively stable, not cumulative over time, and are not related to hyphal fusion events in an established colony. By contrast, mycelial growth arrest is well timed and is not related to an increase in hyphal compartmentation and death. Using a temperature sensitive *het-c* mutant, we determined that HCD could be triggered, in mycelial heterokaryons, after 2 hours incubation at permissive temperatures. Conidia and ascospores, however, are not competent to undergo HCD until 12 to 14 hours after germination. The temperature sensitive *het-c* allele has been cloned and sequenced and is currently being analyzed. Transcriptional analyses of *het-c* revealed a temporal expression in a compatible reaction. Competitive RNA analyses in a reation to Beta-tubulin revealed a peak accumulation of *het-c* transcript at 16 h. These data suggest that vegetative incompatibility mediated by *het-c* is a well-timed and programmed process and is associated with the transition from germination to mycelial growth.

51 **No evidence for endocytosis in *Neurospora* by electron microscopy.** Torralba S., Heath I.B.. York University, Biology, Ontario, CANADA

During tip growth of filamentous fungi, hyphal extension is confined to the hyphal apex and involves highly polarized exocytosis. It is well documented that secretory vesicles deliver membrane, cell wall precursors and wall-building enzymes to the hyphal tip. However, the presence or absence of endocytosis in these organisms is not yet clear. Although endocytosis is well known in animal cells and there is evidence for an endocytotic pathway in yeast and plant cells, reports of the existence of endocytosis in filamentous fungi have been conflicting. Uptake of fluorescent markers such as FM4-64 by living hyphae of various species, including *Neurospora*, has been taken as positive evidence for endocytosis. However, other endocytosis markers were not internalized by hyphae of *Pisolithus* and neither clathrin-coated vesicles nor any other definitive ultrastructural indicator of endocytosis have been found in fungal hyphae. In this work, we have investigated endocytosis in *Neurospora* by electron microscopy, using lanthanum as an opaque endocytosis marker. *Neurospora* hyphae grew at normal rate in the presence of lanthanum. Lanthanum was able to penetrate the cell wall and treated hyphae presented electron dense deposits on the plasma membrane. Some deposits were associated with vesicle-like structures, but such structures were shown to form part of invaginations of the plasma membrane, when followed by serial sections. We failed to observe any internalization of lanthanum into the *Neurospora* hypha and no deposits were present in the cytoplasm or associated

with any organelles, questioning the occurrence of endocytosis, as opposed to molecular internalization of FM4- 64, in this fungus.

52 **Towards an understanding of the mechanisms which generate variability in *Colletotrichum lindemuthianum.*** Raul Rodríguez-Guerra, Maria-Teresa Ramírez-Rueda, June Simpson. CINVESTAV, Unidad Irapuato, Apdo. Postal 629, Irapuato, Gto. Mexico.

Characterization of pathotypes and use of molecular markers has indicated a high level of variability between isolates of *Colletotrichum lindemuthianum*. This variability could be due to spontaneous mutation or sexual or parasexual recombination. To investigate these posibilities, 19 *C. lindemuthianum* isolates from different regions of Mexico were analyzed. Initially all isolates were confronted with themselves and with all other isolates in order to determine their capacity to produce perithecia and/or undergo anastomosis. A small number of isolates produced perithecia when confronted with other isolates however these structures were found to be infertile. A larger number produced spherical structures assumed to be protoperithecia. All isolates anastomosed with themselves but differed in their capacity to anastomose with other isolates. A group of 6 isolates with different capacities for anastomosis was chosen to determine whether new genotypes due to mitotic recombination are formed following confrontation of two distinct isolates. Following confrontation, around 40 spores were isolated individually and subjected to AFLP anaylsis. In confrontations where isolates were capable of anastamosis, new genetic fingerprints different to those of the original isolates were observed. In combinations of isolates unable to anastomose, genotypes corresponded mainly to one of the original isolates. Analysis of 40 individual spores from individual isolates showed identical genotypes, strongly suggesting that the variability observed in genotype patterns is due to mitotic recombination and not to spontaneous mutation. We are grateful to CONACyT (K0195B) and SIHGO

53 **Apoptosis occurs in the Basidia of Mutants of *Coprinus cinereus.*** Benjamin C. Lu[1], Natasha Gallo[1], and U. Kues[2]. [1]University of Guelph, Guelph, Canada. [2]ETH Zurich, Switzerland.

Apoptosis occurs in the basidia of *Coprinus cinereus* when mutations occur that cause defects in (1) meiotic progression, or (2)basidiospore formatino. Mutants that are defective in meiotic progression, such as the assembly of synaptonemal complex, homologous chromosome pairing, or DNA repairs, trigger apoptosis as soon as the meiotic nuclei attempt to enter metaphase I. These mutants will produce some spores varying from extremely low number of tetrad to almost gray cap; the number varies from fruiting body to fruiting body even when they are on the same culture dish. Mutants that are defective in spore formation trigger apoptosis in the tetrad stage after completion of meiosis. Apoptosis follows a set pattern regardless of the type of mutations. It starts with chromatin condensation and DNA fragmentation, followed by plasmolysis, cytoplasmic fragmentation and cell shrinkage of basidia, first in a few cells then gradually spread to nearly all basidia. The end stage is represented by extreme shrinkage and total degradation of DNA of meiotic nuclei. Apoptosis is cell type specific; it occurs only in the basidia and not the surrounding somatic tissues. Apoptosis can be inhibited when meiosis progression is arrested by a checkpoint control that prevents entry to the division program.

54 **Characterization and phylogenetic analysis of the septin gene family from *Aspergillus nidulans* and other fungi.** Michelle Momany, Jiong Zhao, Rebecca Lindsey, Patrick J. Westfall. Department of Botany, University of Georgia, Athens, Georgia 30602

Members of the septin gene family are involved in cytokinesis and the organization of new growth in organisms as diverse as yeast, fruit fly, worm, mouse, and man. We have cloned and sequenced five septins from the model filamentous fungus A. nidulans. As expected the A. nidulans septins contain the highly conserved GTP binding and coiled-coil domains seen in other septins. Hybridization of clones to a chromosome specific library and correlation with an A. nidulans physical map showed that the septins are not clustered, but are scattered throughout the genome. Phylogenic analysis showed that most fungal septins could be grouped with one of the prototypical S. cerevisiae septins, Cdc3, Cdc10, Cdc11, and Cdc12. Intron-exon structure was conserved within septin classes. Examination of asp message levels during asexual spore formation showed that A. nidulans septin gene family members are differentially expressed. Our results suggest that most fungal septins belong to one of four orthologous classes.

55 **A novel putative transmembrane protein is involved in vegetative incompatibility and hyphal fusion in *Neurospora crassa.*** Qijun Xiang and Louise Glass. Department of Plant & Microbial Biology, Berkeley, CA 94720-3102, USA

Vegetative incompatibility triggered by allelic differences at the *het-c* locus in *Neurospora crassa* is characterized by growth inhibition and arrest, suppression of conidiation and hyphal compartmentation and death. To genetically dissect the pathway of *het-c* vegetative incompatibility, we allowed incompatible transformants to escape from growth inhibition and suppression of conidiation. Three mutants were identified that contained a recessive suppressor of vegetative incompatibility, all of which were allelic to the *vib-1* locus, which is located between *lys-2* and *ilv-2* on chromosome V. A common phenotypic hallmark of Vib mutants is that all of them can block *het- c* vegetative incompatibility. Another common characteristic is that all conidiate (nearly) constitutively. *vib-1(2)* and *vib-1(3)* mutants have no other obvious defects, while *vib-1(1)* mutants have additional morphological and developmental defects. The *vib-1(1)* mutant shows aerial hyphae defects and dramatically reduces hyphal fusion events. Crosses between the *vib-1(1)* mutant and wild-type strain show dominant ascospore lethal affects, which may be caused by meiotic transvection. *vib-1* was cloned by complementation of conidiation defect in *vib-1(1)* mutant. It encodes a novel 1085 aa putative protein. Primary analyses indicate that VIB-1 has three putative transmembrane domains. It does not display a high level of identity with proteins of known function in other organisms, except a ~150 aa region in the C-terminal that is similar to a number of hyphothetical proteins in eukaryotic organisms. This conserved region is required for VIB-1 function.

56 **Co-localization of alternative HET-C alleles in hyphae undergoing vegetative incompatibility by confocal microscopy**. Sovan Sarkar and N. Louise Glass. Plant and Microbial Biology Department, University of California, Berkeley CA 94720-3102

In *N. crassa*, vegetative incompatibility is mediated by genetic differences at *het* loci. The *het-c* locus has been previously characterized and initial data indicated that non-self recognition is mediated by the formation of a HET-C heterocomplex. Formation of the HET-C heterocomplex leads to hyphal compartmentation and death (HCD). We demonstrated the co- localization of alternative HET-C proteins in hyphae undergoing vegetative incompatibility by using fluorophore conjugated affinity purified antibodies (cy3 and cy5) to tagged het-c alleles, *het-c* ^{OR}::*GFP* and *het-c* ^{PA}::*HA* using confocal microscopy. Co-localization was observed in the plasma membrane suggesting that formation of the HET-C heterocomplex triggers HCD. Permeabilization studies of cells with 0.1% triton destroyed co-localization indicating that the C-terminus of HET-C is located on the outside of the cell. We have been unable to detect HET-C by itself or in homocomplex. The reason for this is unclear but it maybe due to the instability of HET-C in the absence of heterocomplex formation. HET-C is predicted to have a signal peptide. However, a het-c construct missing the signal peptide triggers vegetative incompatibility, providing one HET-C protein retains a signal peptide. Co-transformation of alternative het-c alleles, both of which lack the signal peptide, resulted in only compatible transformants. Studies to examine the localization of HET-C in signal peptide deleted strains are currently under investigation.

57 **Rapamycin and Tor in the pathogenic fungi *Cryptococcus neoformans* and *Candida albicans*.** Jill Blankenship, M. Cristina Cruz, Maria E. Cardenas and Joseph Heitman. Duke University Genetics Dept. Durham, NC

Cryptococcus neoformans and *Candida albicans* are opportunistic human pathogens that effect primarily immunocompromised patients. Several drug treatments have been developed to combat the serious fungal infections caused by these pathogens, but due to a significant rate of drug resistance and toxicity, novel drug treatments need to be established. The macrolide drug rapamycin is a good candidate for such therapy because it has potent antifungal effects, and non-immunosuppressive rapamycin analogs that retain antifungal activity have been identified. In complex with the protein FKBP12, rapamycin binds to and inhibits the TOR proteins which are conserved from yeast to mammals. Unlike *S. cerevisiae* and *S. pombe*, *C. albicans* and *C. neoformans* have only a single Tor kinase homolog. Thus, these systems provide an opportunity to dissect the functions of Tor that are important for the shared and unique roles of the two Tor homologs in budding and fission yeasts and to examine the role of Tor in pathogenicity and antifungal drug action. We have analyzed the effects of rapamycin on *C. neoformans in vitro* and are determining whether the *TOR1* gene is essential using diploid strains we have recently identified. We have also identified a gene encoding a novel TOR-like kinase Tlk1, which shares significant sequence identity to the Tor

kinase domain but lacks the conserved FKBP-rapamycin binding domain. A *tlk1* mutant strain generated by homologous recombination via biolistic transformation is viable and as yet has no discernable phenotype. Finally, we have constructed a rapamycin resistant *Tor1-1* mutation in *Candida albicans*, and are disrupting the *TOR1* gene in this organism to compare and contrast the functions of the Tor kinases in pathogenic and model yeasts.

58 **Tubular vacuoles in the hyphal tip region of oomycetes.** William G. Allaway and Osu Lilje. School of Biological Sciences, The University of Sydney, NSW 2006, Australia

The tip region of oomycete hyphae contains a network of fine, slow-moving tubular structures which have been identified as tubular vacuoles by their ability to accumulate and retain fluorescent probes. We describe our investigations of the connections of this tubular network in the tip region with larger vacuoles further back from the tip, using fluorescence, DIC and confocal microscopy.

59 Withdrawn

60 **Identification of G1/S regulators which interact physically and genetically with the *Aspergillus nimO*^{Dbf4}protein.** Steve James[1], Carey Connelly[1], Nitin Malhotra[1], Holly Massimilla[1], Karen Messner[2], & Peter Mirabito[3 1]Department of Biology, Gettysburg College, Gettysburg, PA USA. [2]Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC USA. [3]T.H. Morgan School of Biological Sciences, University of Kentucky, Lexington, KY USA.

nimO^{Dbf4} of *Aspergillus nidulans* encodes the regulatory subunit of a conserved eukaryotic enzyme known as Dbf4-dependent kinase (DDK). In budding yeast, Dbf4p initiates DNA synthesis by activating a catalytic subunit, Cdc7p, and escorting it to origins of replication. DDK then triggers origin firing through phosphorylation of DNA helicase subunits. We have identified three Aspergillus genes whose products associate directly with nimOp or indirectly influence its function: (1) the Cdc7^{Asp} homolog was isolated. Overexpression of alcA::Cdc7^{Asp} rescued *nimO*18 ts-lethality, but did not rescue G1/S mutations in other genes. Two-hybrid analysis revealed that N- and C-terminal fragments of nimOp interact specifically with Cdc7p^{Asp}. Furthermore, single amino acid replacements in the C-terminal *nimO* zinc finger abolished the interaction, indicating that this novel motif is essential for nimOp-Cdc7p association; (2, 3) we generated partial *nimO*18 suppressors in two loci, *snoA* and *snoB* (suppressor- of-nimO). The suppressors were tested in a background where the only copy of *nimO* is under the control of the *alcA* promoter. Such strains are normally ethanol-dependent and glucose-inviable. However, recessive *snoA* suppressors appeared to bypass the requirement for *nimO* by rescuing growth on glucose. Semi-dominant *snoB* alleles did not bypass the need for *nimO*, suggesting that snoBp and nimOp may associate directly or act in the same complex. Efforts are underway to isolate *snoA* and *snoB*, and to explore the functions of nimOp and its suppressors by using an epitope-tagged allele of *nimO*. (Supported by NSF-RUI: MCB 95-07485 to SWJ)

61 **Integration profiles of transforming vector in the null mutants of Rad51 homologue in filamentous fungi.** Ichioka Daisuke, Toyoaki Natsume, and Yasuo Itoh Faculty of Science, Shinshu University, Matsumoto, Japan

Some species of filamentous fungi, including the model organism *Aspergillus nidulans*, are useful for studying mechanisms that catalyze the exogenous DNA integration during genetic transformation. Both homologous and ectopic integrations are detected with comparable frequency, in contrast to the bias for either type of integration observed in *Saccharomyces cerevisiae* and animals. To control these types of DNA integration by regulating the catalyzing machinery, we were interested in Rad51 of *S. cerevisiae*. Rad51 has been extensively studied on its involvement in homology search between DNA strands during meiosis and recombinational repair. Site specific disruptants were obtained for *A. nidulans* and *Penicillium paxilli* by gene replacement and integration profiles of transforming vector were analyzed. No homologous integration was detected with a 1.7-kb fragment that targeted for *argB2* locus in the *uvsC* null mutant of *A. nidulans*. Furthermore number of transformants that had ectopic / illegitimate integration at multiple genomic sites was significantly increased by inactivating Rad51 homolog in both species when selection was done with nutrient markers. Geneticin resistance was also used for *P. paxilli* transformation and in this case, evident effect of Rad51 inactivation was decreased occurrence of direct repeat formation. These results were consistent with the established function of Rad51. Increased number of integration

events by *RAD51* disruption could be explained by the inefficiency of double-strand break repair apparatus that does not require

62 **Genetic analysis of the calcineurin signal transduction pathway in *Cryptococcus neoformans.*** Deborah S. Fox and Joseph Heitman. Departments of Genetics, Pharmacology and Cancer Biology, Microbiology, and Medicine, and the Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC

Cryptococcus neoformans is an opportunistic fungal pathogen that causes life-threatening meningoencephalitis in immunocompromised patients. Calcineurin is a Ca^{2+}-calmodulin-activated protein phosphatase and a target of the immunosuppressive drug FK506, which inhibits calcineurin activity. In other organisms, calcineurin regulates cell polarity, mating, and cytokinesis. Previously, we have shown that disruption of the genes encoding the catalytic or regulatory subunits of calcineurin in *C. neoformans* abolishes growth at elevated temperature, virulence, hyphal elongation, and haploid filamentation. In this study we examined the hypothesis that calcineurin-dependent effectors are present in *C. neoformans*, and that their over-expression will suppress the temperature-sensitive growth defect conferred by a calcineurin mutation. To identify components of the calcineurin signaling pathway in *C. neoformans*, we isolated multicopy suppressors of the temperature-sensitive defect of a calcineurin-deficient strain. A multicopy genomic library was introduced into a calcineurin mutant strain. Plasmid-dependent transformants were isolated which restored growth at high temperature, revealing a novel gene, named *CTS1* for calcineurin temperature suppressor. *CTS1* encodes a C2 domain- containing protein, and may function as an effector of calcineurin. Over-expression of Cts1 conferred resistance to FK506 in wild-type organisms and suppressed the temperature-sensitive phenotype of calcineurin mutant strains. Disruption of the *CTS1* gene by homologous recombination resulted in a cytokinesis defect, synthetic lethality with calcineurin mutations, enhanced sensitivity to FK506, and a reduction in mating and haploid filamentation. Ongoing studies aim to elucidate the functions of Cts1 in the calcineurin signal transduction pathway and may further our understanding of the roles of calcineurin in virulence and differentiation.

63 **Isolation and characterization of the *npgA* gene involved in pigment formation in *Aspergillus nidulans*.** Jung-Mi Kim, Dong Min Han[1], Keon-Sang Chae, Suhn-Kee Chae[2], and Kwang-Yeop Jahng. Division of Biological Sciences, Chonbuk National University, Chonju, South Korea; [1]Department of Molecular Biology, Wonkwang University, Iksan, South Korea; [2]Division of Life Science, Paichai University, Taejon, South Korea.

The *npgA* gene has been known that it plays an important role in constructing cell wall structure or depositing pigment in *Aspergillus nidulans*. To study the biological function of *npgA* gene, we isolated and sequenced the nucleotide of the DNA fragment that complemented *npgA1* mutation from the genomic cosmid library of *Aspergillus nidulans*. Sequence data of the full-length *npgA* showed that there was an open reading frame (ORF) possibly encoding a polypeptide of 344 amino acid. The amino acid sequence showed 30-40% similarity to the amphipatic protein surfactin of *Bacillus subtilis*. The sequence analysis revealed that the *npgA* is linked to the gene *aatA* encoding the homolog of acyl transferase on the chromosome 1. The 269th codon (TTA) for leucine of wild type ORF was replaced by termination codon (TGA) in the *npgA1* mutation. The transcript of *npgA* gene appeared throughout the whole life cycle. The deletion mutant of *npgA* neither grew well nor showed normal pigmentation throughout the whole life cycle suggesting that the full length of NpgAp might be required for the celllular growth as well as metabolite formation. However over-expression mutant of *npgA* showed no distinguishable phenotype in growth and pigmentation except the conidiopores formation was earlier. The protoplasts were generated more rapidly and in larger quantity from *npgA* deletion mutant. The viscosity of culture filtrate of *npgA* deletion mutant was similar to *npgA1* mutant. These phenotypes of deletion mutant and the amino acid homology to the surfactin imply that the protein NpgAp might be involved in the synthesis of secondary metabolites such as pigment in *A. nidulans*.

64 **Isolation and characterization of two genes encoding mitogen-activated protein kinase homologs, *mpkB* and *mpkC*, from *Aspergillus nidulans*.** Sei-Jin Lee, Hyun-Joo Park, Dong Min Han[1], Keon-Sang Chae, Suhn-Kee Chae[2], and Kwang-Yeop Jahng. Division of Biological Sciences, Chonbuk National University, Chonju, South Korea; [1]Department of Molecular Biology, Wonkwang University, Iksan, South Korea; [2]Division of Life Science, Paichai University, Taejon, South Korea.

Mitogen-activated protein kinases (MAPKs) play an important role in regulation of diverse cellular functions such as growth, differentiation, osmoregulation and cell wall biosynthesis in fungi. Two genes encoding mitogen-

activated protein kinase homologs, *mpkB* and *mpkC*, were isolated from *Aspergillus nidulans* by PCR with degenerated primers and colony hybridization. Each nucleotide sequence of *mpkB* and *mpkC* consisted of presumptive ORF containing 457 and 347 amino acids and 3 and 6 introns, respectively. The amino acid sequence of MpkBp showed 84% of identities to the gene *pmk1* which is involved in differentiation and pathogenicity in *Magnaporthe grisea* suggesting that the gene *mpkB* might play some roles in development in *A. nidulans*. However the amino acid sequence of MpkCp showed 50% of identities to the gene *HOG1*, which was known to regulate the osmostress in yeast, implying that the gene *mpkC* might be a member of stress-activated protein kinases (SAPK). To identify and characterize the function of the gene *mpkB*, it was disrupted and over-expressed. The phenotype of disruptant of *mpkB* showed not forming of cleistothecium when induced sexual development. However over-expression mutant did not show any phenotypic changes. From these results, we suggest that the MAPK gene of *A. nidulans*, *mpkB* should be required for the formation of sexual organ, cleistothecium. In contrast, the deletion mutant and over-expression mutant of *mpkC* showed no significant phenotypes in the various conditions of environmental stress. From our results, we learned that MAP kinases are possibly involved in sexual development in *A. nidulans*.

65 **Cellular function of a gene encoding G protein alpha subunit homolog in the development of *Aspergillus nidulans*.** Mi-Hee Chang, Dong Min Han[1], Keon-Sang Chae, Suhn-Kee Chae[2] and Kwang-Yeop Jahng. Division of Biological Sciences, Chonbuk National University, Chonju, South Korea; [1]Department of Molecular Biology, Wonkwang University, Iksan, South Korea; [2]Division of Life Science, Paichai University, Taejon, South Korea.

Signal transduction via G protein coupled receptor plays important roles in various cellular responses in eukaryotic organism. Recently many fungal G proteins have been identified in various species and most of them are involved in mating, development, growth and morphogenesis of fungi. In *Aspergillus nidulans*, a G alpha gene, *fadA* has been known to have a major role in determining the balance between growth and sporulation. We have previously isolated the gene *ganA* that presumably encodes G protein alpha subunit homolog from *A. nidulans*. However its biological function is not clear yet. Here we present that another G protein alpha subunit homolog, *ganB*, is involved in growth and development in *A. nidulans*. Putative GanBp is consisted of 356 amino acids and closely related to Gs alpha subfamily. It has consensus myristoylation site at amino terminal. Transcription of *ganB* was likely to be dependent on the developmental stage. The transcript of *ganB* increased as sexual development was proceeded. To investigate cellular function of *ganB* we constructed various mutants of *ganB* by site-specific targeting. Constitutive activating mutation of *ganB* significantly reduced asexual sporulation and led abnormal mycelium growth.

66 **Characterization of secretion related small GTPase encoding genes in *Aspergillus niger*.** X.O. Weenink[1], A.F.J. Ram[1,2], B. Seiboth[3], B. Luken[2], P.J. Punt[2] and C.A.M.J.J. van den Hondel[1,2]. 1)Leiden University, Inst. Mol. Plant Sciences/Centre for Phyto-techn., Wassenaarseweg 64, 2333 AL, Leiden, The Netherlands, 2) Dep. Appl. Microbiol. Gene Techn., TNO-Nutrition, 3700 AJ Zeist, The Netherlands, 3)TU Wien, Inst. for Biochem. Microbiol., Wien, Austria.

Filamentous fungi, including *Aspergillus niger*, have the capacity to secrete large amount of enzymes into their environment. Transport of these proteins through the secretion pathway is mediated by vesicles. The formation, budding and fusion of vesicles is regulated by highly conserved secretion related GTP-binding proteins. Based on this conservation, we have cloned seven **s**ecretion **r**elated small **G**TPases (*srg* genes) from *A. niger*. Transcript analysis of the different GTPases revealed unusually long 5ÆÆ and 3ÆÆ untranslated regions for all *srg* genes. Expression of the several *srg* genes was independent of different carbon sources used. Multiple transcripts were detected for *srgA*, *srgC*, *srgE* and *sarA*. The largest transcripts of *sarA*, *srgC* and *srgE* were abundant throughout different stages of growth, whereas their smaller transcripts were only detected during early growth. This latter behaviour was also found for *srgD* and *srgF*. In contrast, both *srgA* transcripts and the single *srgB* transcript were abundant throughout the whole cultivation period. We have started to make mutants (deletion or conditional) to systematically inactivate the various srg genes to generate a collection of secretion mutants with defined blocks in the secretion pathway. To monitor protein secretion both in wild type and various secretion mutants strains we have deleveloped in a GFP- based secretion reporter system which is used for the analysis of the secretion mutants.

67 **The roles of *CHS1*, *CHS8* and *BNI4* in cell wall synthesis in *C. albicans*.** Munro, C.A., Winters, K., Rella, M., Rowbottom, L., Bulawa, C.E. and Neil A.R. Gow. Dept of Molecular and Cell Biology, University of Aberdeen, Scotland, UK. Millenium Pharmaceuticals, 75 Sidney Street, Cambridge, MA, US.

Chitin is a critical component of fungal cell walls. Here we present recent advances in the molecular analysis of chitin synthase in the human pathogen *Candida albicans*. In this fungus there are 4 chitin synthases. We have shown recently that CaChs1p synthesizes the septal chitin and contributes to chitin in the lateral cell wall. A conditional delta*chs1* mutant was generated by regulating the expression of *CaCHS1*. Under repressing conditions yeast cells no longer separated from each other after budding and grew as septum-less chains for several generations before growth ceased. Hyphae grew for several hours with a normal morphology. Eventually hyphae stopped growing, the cell wall ballooned at various positions and cells lysed. *CaCHS1* is the first example of an essential chitin synthase in fungi. CaChs2p encodes the major chitin synthase activity *in vitro*, and delta*chs2* null mutants have marginally less chitin in hyphal cells. CaChs3p synthesizes the majority of cell wall chitin and the ring of chitin at the site where a new bud emerges. Recently we identified a fourth gene encoding a chitin synthase in *C. albicans* we have named this *CHS8* (Chs4-7p are apparently regulators of Chs3p). CaChs8p has the highest identity to CaChs2p at the amino acid level. The function of CaChs8p is currently under investigation and latest progress will be presented . Several proteins have been identified as regulators of Chs3p in *S. cerevisiae*. One regulator Bni4p is found at the mother-bud neck associated with septins and Chs4p, which in turn is linked to Chs3p. A homologue of *BNI4* was identified in *C. albicans*. The delta*Cabni4* null mutant has a 30% reduction in cell wall chitin compared to wild type cells. Calcofluor staining revealed the chitin ring was still synthesized at the bud neck and bud scars were evident suggesting that CaBni4p has a redundant function in targeting Chs3p to the mother-bud neck in *C. albicans*.

68 **Quantitative Analysis of the expression of ABC-transporter genes in *Aspergillus nidulans* by Real-Time RT-PCR Assay.** Camile P. Semighini, Marcelo A. Vallim, Mozart Marins, Renata Castiglioni Pascon, Maria Helena de Souza Goldman[1], and Gustavo Henrique Goldman. Faculdade de Ciências Farmaceuticas de Ribeirão Preto and [1]Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Brazil

The frequency of life-threatening fungal infections is rising world-wide. Drug treatment failures in fungal infections combined with improvements in performance and standardisation of antifungal-susceptibility testing have drawn attention to the problem of antifungal drug resistance. It is now clear that antifungals can create clinical and epidemiological situations that are analogous to those found with antibiotic-resistant bacteria. Typical gene products responsible for multiple drug resistance (mdr) are ABC (ATP-Binding-Cassette) transport proteins which are responsible for the efflux of toxic compounds. Here, we evaluate the potential of real-time RT-PCR to quantify the mRNA expression of four ABC-transporter genes from *A. nidulans*, named *AtrA, AtrB, AtrC* and *AtrD*. The *Atr*-genes were shown to be induced by several structurally different drugs, a hallmark of the mdr phenotype. These genes displayed a very complex expression pattern in different *ima* genetical backgrounds. The *imaB* mutant has a higher basal level of expression of *AtrB* and *-D* than the wild-type strain and these two genes have comparable levels of expression when the *imaB* mutant is grown in the presence and absence of imazalil. This dependence between the presence of *imaB* and expression of *AtrB* and *-D* indicates that the *imaB* locus regulates *AtrB* and *-D* expression, possibly acting as a a regulatory gene. The *imaB* mutant has a higher basal level of expression of *AtrB* and *-D* than the wild-type. These results showed a complex net of interactions among expression levels of the different ABC-transporter genes in the ima mutants background. Quantitative analysis of the mRNA expression of these genes in multidrug-resistant of clinical isolates, will allow us to access their behavior in several pathogenic fungi leading to the improvement of antifungal therapy. Financial support: FAPESP-Brazil

69 **Molecular analysis of three kinesins in *Aspergillus nidulans*.** Natalia Requena, Patricia Rischitor, Else Winzenburg, Ralf Liese and Reinhard Fischer. Max-Planck-Institut for terrestrial Microbiology, Karl-von-Frisch-Str., D-35043 Marburg, Germany.

Motor proteins are involved in a variety of different cellular processes such as mitosis, cytokinesis or organelle movement. In A. nidulans and several other fungal species dynein is required for nuclear migration. Recently, it was shown that in *S. cerevisiae* in addition to dynein, several kinesins are involved in nuclear positioning and nuclear migration. Since the processes of nuclear migration could be rather different in yeast cells and in filamentous fungi, we wanted to investigate the roles of different kinesins in *A. nidulans*. Taking advantage of the *A. nidulans* sequencing project at Cereon Genomics (Cambridge, USA) several partial kinesin sequences were obtained. Further sequence analysis revealed that one of them displayed strong homology to conventional kinesin identified before in several fungi. The complete sequence was obtained, the gene (kinA) deleted and the kinesin deficient strain analysed for vesicle, mitochondrial and nuclear movement using the GFP technology. We present evidence that this motor protein affects the stability of microtubules. In addition, homologues of *S. cerevisiae* Kip2 and Kip3, both of which

are involved in nuclear migration, are studied. Deletion strains of the corresponding genes in A. nidulans were constructed as well as double and triple mutants. These strains are phenotypically analysed with respect to nuclear migration and microtubule stability.

70 **The effects of ropy-1 mutation on cytoplasmic organization and intracellular motility in mature hyphae of *Neurospora crassa*.** Robert Roberson. Arizona State University Plant Biology

To better understand the role(s) of dynein during hyphal tip growth, we have used light and electron microscopy to document the cytoplasmic effects of the ropy (ro-1) mutation in mature hyphae of Neurospora crassa. Transmission electron microscopy (TEM) showed that the apical cytoplasm of wild-type hyphae contained a typical Spitzenkörper (Spk) composed of a spherical aggregation of secretory vesicles ranging in size from 120 (macrovesicles) to 50 (microvesicles) nm diam. In most Spk, these vesicles surrounded a central core composed primarily of microvesicles embedded in a dense granular/fibrillar matrix. ro-1 hyphae contained Spk of reduced size and unclear pattern of vesicle distribution. Video-enhanced light microscopy was used to characterize intracellular behavior of multivesicular bodies (MVBs) and mitochondria in wild-type and ro-1 hyphae. In wild-type cells, MVBs traveled along paths that were generally parallel to the longitudinal axis of the cell. Anterograde and retrograde motility was observed, with most travel being anterograde. A small population of mitochondria displayed rapid anterograde and retrograde movements, while most maintained a constant position relative to either the advancing cytoplasm. In ro-1 hyphae, the motility and/or positioning of MVBs and mitochondria was significantly altered relative to the wild-type. Immunofluorescence confocal microscopy revealed that the microtubule cytoskeleton was severely disrupted in the ropy mutant. Clearly, dynein deficiency in the mutant causes profound perturbation on cytoskeleton organization and organelle dynamics. These perturbations impact negatively on the organization and stability of the Spk, which, in turn, leads to severe reduction in growth rate and altered hyphal morphology.

71 **G gamma subunit homolog identified in *Neurospora crassa*.** S. Krystofova, K.A. Borkovich. University Texas HSC Houston, Dept. Microbiology and Molecular Genetics, Texas, USA.

Heterotrimeric guanine nucleotide-binding proteins (G proteins) consisting of alfa, beta and gamma subunits mediate signalling between cell surface receptors and intracellular effectors in eukaryotic cells. Upon agonist binding to the receptor, the G protein alpha subunit releases GDP, binds GTP, and dissociates from the G protein beta gamma subunit dimer. Depending on the system, either G alfa or G beta gamma go on to activate downstream effectors. In *Neurospora crassa* it is a G alfa homolog (GNA-1) that positively regulates adenylyl cyclase activity. Three G alfa subunits genes, gna-1, gna-2 and gna-3, and one G beta subunit gene, gnb-1, have been identified in Neurospora crassa. We identified a putative G protein gamma subunit, gng-1, in N.crassa during homology searches of the University of Oklahoma EST database. The full length of gng-1 cDNA (273bp) has been found in the cDNA clone. Our data have shown that the introns are in positions which are conserved in the mammalian G gamma subunits. The putative protein sequence of GNG-1 shows a COOH-terminal CAAX motif of isoprenylation commonly found in G gamma subunits (CVVM). GNG-1 is closely related to *Saccharomyces cerevisiae* G gamma subunit, STE18 (35% identity) and non-visual mammalian G gamma subunits (30-33% identity).

72 **Live cell imaging of hyphal fusions in growing Neurospora colonies** Patrick C. Hickey[1], David J. Jacobson[2], Nick D. Read[1] and N. Louise Glass[2]. [1] Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, EH9 3JH, UK. [2] Department of Plant and Microbiological Science, University of California at Berkeley, California 94720, USA.

The process of hyphal fusion (anastomosis) in growing colonies of Neurospora has been visualized using confocal microscopy. A variety of fluorescent dyes were used to follow different cell components during anastomosis. These include the plasma membrane, the Spitzenkörper, mitochondria, vacuoles, and nuclei. Time-lapse movies illustrate the dynamics of these cell components in each of the discrete physiological states of the participating hyphae: pre-contact, post-contact, and post-fusion. Fusion-competent hyphae were generally morphologically distinct and often showed forms of remote sensing, resulting in branch initiation and re-direction of growth to facilitate contact. Intense membrane activity was seen where fusion-competent hyphae met (post-contact), possibly due to vesicles delivering compounds to first agglutinate cell walls at the point of contact and subsequently to soften cell walls only at the fusion pore. Three-dimensional reconstruction of confocal images clearly showed the structural changes of hyphae at the fusion pores. De novo septum formation, at a distance from the fusion site, often accompanied

anastomosis. Dramatic post-fusion changes in cytoplasmic flow were frequently visible. The role of anastomoses in the dynamics of cytoplasmic flow in the mycelium is being assessed. Taken together, these observations form a mechanistic model of the events accompanying anastomosis. Imaging of mutants with known defects in hyphal fusion will be used to test this model. In addition, post-fusion events in compatible anastomoses provide the framework for future studies of programmed hyphal compartmentation and death caused by the genetically controlled vegetative incompatibility system.

73 **Characterization of MKK1, a putative MAP kinase kinase in *Pneumocystis carinii*.** Donald J. Ferguson Jr. and A. George Smulian. VA Medical Center, Cincinnati, OH.

Signal transduction pathways in various fungi have been extensively studied. However, much remains to be elucidated regarding the signal transduction pathways of the opportunistic fungal pathogen *Pneumocystis carinii*. Recently, the gene encoding a putative mitogen-activated protein kinase kinase (MAPKK or MEK) referred to as mkk1, was cloned and is predicted to encode the MEK in the stress response signal transduction pathway previously defined in *P. carinii*. Sequence alignments displayed homology between the *P. carinii* MKK1 and the known MEK homologs of *Saccharomyces cerevisiae*. Sequence homology predicts that MKK1 would complement a mkk1deletion/mkk2deletion defect in the cell integrity pathway of *S. cerevisiae*. The wild type MKK1-GST was unable to complement the pbs2deletion defect in the high osmolarity growth pathway or the ste7deletion defect in the pheromone response pathway of either mating type a or type alpha *S. cerevisiae.* Site directed mutagenesis was used in an attempt to generate constitutively active and inactive mutants of MKK1. The wild type MKK1 as well as the mutants were cloned into a modified pYX 213 yeast expression vector containing a TPI promoter and a glutathione-S- transferase (GST) tag. Western blotting using anti-GST antibodies revealed products of the appropriate size to indicate expression of the GST-fusion Mkk1 proteins. The MAP kinases MKP1 and MKP2 of *P. carinii* have been cloned and expressed in our laboratory and will be examined for their ability to serve as substrates for the *P. carinii* MKK1.

74 **Isolation of sepA enhancer mutations in *Aspergillus nidulans*.** Claire L. Pearson and Steven D. Harris. Dept. of Microbiology, University of Connecticut Health Center, Farmington, CT.

In *Aspergillus nidulans*, the sepA gene is required for the formation of septa and polarized morphogenesis. SEPA is a member of the formin family, which is characterized by the presence of multiple functional domains that mediate interactions with other proteins involved in organization of the actin cytoskeleton. The goal of this project is to identify proteins that interact with SEPA or, function in parallel pathways. To identify these proteins, we have initiated an enhancer screen using a strain in which the only copy of sepA is expressed under the control of the inducible alcA promoter. To date, we have identified ten mes (morphological defective enhancer of SEPA) mutants that display severe morphological defects on repressing media. One of the mes mutations (mesA1) causes pronounced defects in hyphal morphogenesis in a wildtype background. We have demonstrated that this mutation also enhances the phenotypes caused by the Ts sepA1 allele, which suggests that mesA and sepA may function in parallel genetic pathways to control tip growth in *A. nidulans.* The mesA1 mutant displays defects in the maintenance of hyphal polarity and in the pattern of cell wall deposition. We have mapped mesA1 to chromosome II, and have identified a genomic clone that appears to complement the morphological defects. Preliminary sequence analysis of this clone reveals homology between mesA and an uncharacterized gene in *Schizosaccharomyces pombe*. Further characterization of this clone, as well as the phenotypes caused by the mesA1 mutation, will be presented.

75 **Characterization and localization of the *Aspergillus nidulans* Formin SEPA.** Kathryn E. Sharpless and Steven D. Harris. Dept. of Microbiology, University of Connecticut Health Center, Farmington, CT 06030-3205

SEPA is a member of the formin family of proteins, which are thought to act as molecular scaffolds that organize signaling and structural proteins involved in actin-dependent processes. In *Aspergillus nidulans,* SEPA is required for actin ring formation at septation sites, and is also involved in polarized tip growth. SEPA is thought to organize actin filaments in septal rings and at hyphal tips by interacting with other proteins through multiple distinct domains, including the conserved FH1, FH2, and FH3 domains. To identify functionally important domains in SEPA, we determined the sequence of the temperature sensitive sepA1 and sepA3 alleles. The sepA1 mutation maps to a conserved residue in the FH2 domain, whereas the sepA3 mutation lies in a region of SEPA that potentially binds Rho family GTPases.

To further characterize SEPA, a functional SEPA::GFP fusion protein has been constructed and localized to both septation sites and hyphal tips. Using live imaging, we have found that SEPA forms a ring that constricts and disappears at septation sites. Simultaneously, at hyphal tips, SEPA is found as a dynamic dot or crescent-shape patch. We have also determined that SEPA co-localizes with actin at both septation sites and hyphal tips. Furthermore, we have determined that the amino terminus of sepA is sufficient for the localization of SEPA to both sites, and that the sepH gene product is required for SEPA localization at septation sites but not for localization at hyphal tips.

76 **Isolation of a white collar-1 homolog gene from *Trichoderma harzianum.*** Rios-Momberg, Mauricio; Bibbins, Martha and Herrera-Estrella, Alfredo. Departamento de Ingenieria Genetica, CINVESTAV, Irapuato, Mexico

The biological control agent *Trichoderma harzianum* responds to blue light forming a ring of green conidia at what had been the colony perimeter at the time of the light pulse. The action spectrum of this phenomenon is similar to the absorption spectrum of flavins and to that of many responses to blue/UV-A light in several species of fungi, to that of photomorphogenesis of lower plants and to that of higher plants phototropism. The photoreceptor for phototropism is a LOV domain-flavin based protein, as the white collar-1 protein, the putative blue light photoreceptor of *Neurospora crassa*. In order to determine its possible role in the Trichoderma photoresponses, we describe in this work the isolation and sequence analysis of a white collar-1 homolog gene from Trichoderma harzianum. The putative protein is 150 aa shorter and 60% identical to WC1 over the 800 aa carboxi-terminal region spanning the LOV, PAS and zinc-finger domains.

77 **Suppression of ATM kinase defects by mutation of a RecQ helicase in *Aspergillus nidulans.*** Amy F. Hofmann and Steven D. Harris. Genetics, Molecular Biology and Biochemistry, Department of Microbiology

The *Aspergillus nidulans* uvsB gene encodes a member of the conserved family of ATM-related PI-3 kinases. Members of this family of proteins function as central regulators of the DNA damage response. Mutation of the human ATM gene results in the cancer prone syndrome ataxia telangiectasia. In *Aspergillus nidulans*, UVSB is required for multiple aspects of the DNA damage response, including, 1.) arrest of nuclear division, 2.) inhibition of septation, 3.) damage induced transcription, and 4.) induction of mutagenesis. The *Aspergillus nidulans* musN227 and musP234 mutations were originally isolated in a screen for mutants sensitive to methyl methane sulfonate (MMS) (Kafer and Mayor 1986). Both musN227 and musP234 are capable of partially suppressing the poor growth and DNA damage sensitivity of uvsB110 mutants (Kafer and Chae 1994). We have shown that the musN227 mutation partially restores several of the uvsB110 defects, suggesting that MUSN may play a role in recovery from the DNA damage response. musN227 is also capable of partially suppressing the damage sensitivity of a uvsB null mutant, indicating that the suppression is not dependent on any UVSB function. Preliminary results suggest that overexpression of MUSN increases the sensitivity of wildtype to genotoxic agents. This is consistent with a role of MUSN in recovery, as overexpressing MUSN may cause premature recovery before cells have adequately repaired or replicated their DNA. Molecular characterization of the musN gene indicates that it encodes a member of the RecQ family of helicases. These helicases appear to play an important role in maintaining genomic stability in eukaryotic organisms. This is exemplified by the fact that mutations in two related human genes, BLM and WRN, cause a cancer prone syndrome and an aging syndrome respectively. Members of the RecQ helicase family of proteins are reported to be involved in the resolution of Holliday junctions via branch migration. The role of MUSN in recovery from the DNA damage response may be associated with this function. The identity of MUSP remains unknown; however, it appears that expression of musN is capable of partially rescuing the damage sensitivity of the musP234 mutant.

78 **Structural analysis of Hsp30, the small heat shock protein of *Neurospora crassa*.** Nora Plesofsky and Robert Brambl. Department of Plant Biology, University of Minnesota, St. Paul, MN 55108

The alpha-crystallin-related heat shock proteins (hsps) comprise an important group of chaperones that are synthesized by all eukaryotic and prokaryotic organisms. These small hsps have been found to strongly enhance resistance to biological and chemical stresses, although they are not essential for survival at normal temperature. We earlier found by targeted mutagenesis that Hsp30 was required for Neurospora crassasurvival at high temperature under conditions of glucose limitation. Furthermore, the mutant cells were strikingly reduced in their glucose phosphorylating activity and in their ability to import proteins into mitochondria. Most small hsps self-assemble into

multimeric particles, and this ability to oligomerize correlates with their chaperone activity. We approached the question of how small hsp monomers might be organized in these structures by testing and measuring the binding interactions between pairs of small Hsp30 peptides, using the yeast two-hybrid system. We found that sequences within the most conserved region of these small hsps, in the C-terminal "alpha-crystallin domain," interact with two different domains of Hsp30. There is strong interaction of this conserved region with the complete N-terminal half of Hsp30 and with the complete C-terminal half. Further refinement of these peptide interactions shows that non-identical portions of the conserved domain are required for the N-terminal and the C-terminal interactions. Both conserved and nonconserved sequences within the N-terminal half of Hsp30 are required for interaction with the conserved C-terminal domain. We believe these peptide interactions, detected by two-hybrid assay, may provide the bases for dimerization and oligomerization that characterize small hsps. The involvement of nonconserved sequence in these interactions might provide a basis for the specificity of multimerization for a particular monomer.

79 **A heterocomplex formed by alternative HET-C proteins triggers vegetative incompatibility in *Neurospora crassa*.** Gopal Iyer[1], Jennifer Wu[2] and Louise Glass[1]. [1]111, Koshland Hall, Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720. [2]Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, WA 98109.

The rejection of the nuclei in a heterokaryotic fusion cell is controlled by the multiallelic het-clocus. Non-self recognition is triggered by allelic differences at het-clocus, which results in growth inhibition. The introduction of alternative het-callele epitope-tagged constructs by co- transformation into het-cnull strain conferred an incompatible phenotype. Co-immunoprecipitation experiments linked the formation of a heterocomplex of alternative HET-C polypeptides with hyphal compartmentation and death response. Further evidence using organelle fractionation localized the heterocomplex to the plasma membrane. This observation was consistent to the in silico analysis predicted for the HET-C polypetide, which revealed the presence of two transmembrane domains. These findings support a possible model of a signal transduced by HET-C, that initialed the formation of a heterocomplex triggers early biochemical reactions that are essential for hyphal compartmentation and death and activation of unidentified effector machinery.

80 **Analysis of the *Cochliobolus heterostophus* homologs of yeast VAC8 and FAB1.** Natalie L. Catlett, Olen C. Yoder, and B. Gillian Turgeon. Novartis Agricultural Discovery Institute, San Diego, California

The fungal vacuole is important for protein degradation, ion and small molecule storage, and osmoregulation. To investigate the role of the vacuole in pathogenesis and developmental processes in filamentous fungi, we disrupted homologs of S*accharomyces cerevisiae* VAC8 and FAB1 in the corn pathogen *Cochliobolus heterostrophus.* In yeast, the armadillo-repeat protein ScVac8p is required for several nonessential vacuolar membrane processes including vacuole-vacuole fusion, cytoplasm to vacuole protein targeting, and transport of the vacuole to the bud. ScFab1p is responsible for production of phosphatidyl inositol 3,5-bisphosphate (PI3,5P2), which is required for normal vacuole morphology and function in yeast. Both the *C. heterostrophus* vac8 and fab1 deletions are viable and produce viable conidia. Analysis of Chvac8 and Chfab1 deletion strains with the vital dye FM4-64 failed to reveal the gross morphological abnormalities of the vacuole seen in the corresponding yeast mutants. Effects of these deletions on plant pathogenesis and mating are under investigation.

81 **SEPB of *Aspergillus nidulans* is a conserved protein which functions to maintain genome stability.** Scott E. Gygax, Amy Hofmann, and Steven D. Harris. Dept. of Microbiology, University of Connecticut Health Center, Farmington, CT. 06030-3205.

A temperature sensitive mutation in the *Aspergillus nidulans* sepB gene dramatically perturbs chromosomal DNA metabolism. At restrictive temperature, this mutation causes; i) elevated levels of mitotic recombination, ii) enhanced levels of chromosomal loss, iii) progressive delays in nuclear division, and iv) the formation of morphologically aberrant interphase nuclei. Molecular characterization of the sepB gene demonstrates that it encodes a 837 amino acid predicted protein possessing five N-terminal consensus WD-40 repeats, a C-terminal Helix-Loop-Helix motif, and a motif shared with DNA polymerase a. Upon sequencing the sepB3 mutant allele, we identified a single missense mutation (P618S) in a conserved proline residue preceding the C- terminal HLH motif, suggesting that the structural stability of this motif might be essential to its function. Protein sequence homology suggests that SEPB might be a member of a conserved family of proteins (*S. cerevisiae* Ctf4p, X. laevis AND-1, and

H. sapiens hAND-1) required for the maintenance of genome stability. ctf4 mutants have a similar genome instability phenotype, and it has been shown that Ctf4p physically binds to DNA polymerase a suggesting a replication function. We are currently assessing the level of functional conservation within the SEPB family of proteins by testing the ability of hAND-1 and Ctf4p to complement the sepB3 mutant.

Phenotypic characterization of sepB3 double mutants in backgrounds defective in the DNA damage checkpoint (uvsB110, uvsD153, nimX^{cdc2AF}) or DNA recombination (nuv2, nuv4, nuv8, and musN227) revealed several synthetic interactions. The sepB3 mutation also causes an elevated level of both spontaneous and induced mutagenesis at the permissive temperature. Phenotypic characterization of sepB3 double mutants with a hyper-recombinatory mutation such as musN227 (RecQ helicase) demonstrated an increase in mutagenesis compaired to sepB3 alone suggesting that the increased mutagenesis is dependent upon recombination. At semi-permissive temperature, the sepB3 mutation causes enhanced sensitivity to MMS and bleomycin. These data suggest that the SEPB protein family might have a role in DNA replication during S-phase as well the repair of double-strand breaks (DSBs).

82 **The vacuolar ATPase in *Neurospora crassa*: Structure of the enzyme and phenotype of strains that lack specific vacuolar ATPase subunits.** Christopher Chavez, Karen Tenney, Emma Jean Bowman, and Barry Bowman. Department of Molecular Cell and Developmental Biology, University of California, Santa Cruz, CA 95064

The vacuolar ATPase is a large multisubunit enzyme that generates an electrochemical gradient for protons across several types of cell membranes. We have identified the genes that encode all 13 of the known subunits of this enzyme. The function of these subunits and a model for their position in the enzyme will be presented. We have also used the RIP procedure to generate strains in which genes for five of the subunits (vma-1, 3, 5, 13, and vph-1) have been inactivated. Inactivation of these genes causes severe morphological changes and also alters the structure of vacuoles within the cell. A characteristic phenotype of strains that lack vacuolar ATPase is the inability to grow in alkaline medium. We have found that mutations in genes that do not appear to encode components of the ATPase can suppress this pH-conditional growth phenotype.

83 **Evidence for activation of MAP kinases during vegetative incompatibility in *Neurospora crassa*.** Amita Pandey and Louise Glass. Plant and Microbial Biology Department, University of California, Berkeley, CA 94720-3102

Filamentous fungi exist as a network of hyphal filaments that can undergo frequent fusions within or between individual colonies. After hyphal fusion between genetically dissimilar individuals, heterokaryon formation is restricted by genetic differences at het loci. Hyphal fusion cells are quickly compartmentalized and undergo hyphal compartmentation and death. Phenotypic aspects associated with HCD are similar in various het interactions, which suggests activation of common signaling pathways. MAP kinases have been implicated in cell proliferation, cell cycle arrest and cell death in various organisms. The main objective of the study is to find out the role of MAP kinases in vegetative incompatibility in Neurospora. From the western blot data we show that both ERK1 and ERK2 homologs are phosphorylated when two isolates differing at either het or mat locus form an incompatible heterokaryons. A mutant containing a deletion in the putative erk-2 homolog, mak-2 (kindly provided by P. Bobrovich and D. Ebbole) was assessed for its role in vegetative incompatibility. The mak-2 mutant shows a hyphal fusion defect, is female sterile and has non-repressible conidiation. The mak-2 mutant is missing the erk-2 homolog as observed in the western blots using mammalian ERK1/ERK2 antibodies. We have also used a modified heterokaryon test and have priliminary data that suggests the involvement of MAK-2 in vegetative incompatibility mediated by differences at the mating-type locus. Transformations and protoplast fusion experiments are underway to confirm the role of MAK-2 in mating type and het-c vegetative incompatibility.

84 **Characterization of vesicle subsets in CHV1 infected *C. parasitica***. Massimo Turina, Patricia McCabe, Antonio Prodi, and Neal Van Alfen. UC Davis, Plant Pathology

The filamentous ascomycete *Cryphonectria parasitica*, the causal agent of chestnut blight, is stopped in its development by Cryphonectria parasitica hypovirus 1 (CHV1) infection, resulting in hypovirulent strains of the

fungus. Previous studies on the cytopathological effects of virus infection on its host cells showed a consistent proliferation of host vesicles so far uncharacterized, where virus replication and dsRNA accumulation occur. Isopicnic D_2O-Ficoll gradients were used to separate subsets of vesicles from the microsomal fraction of virus infected and uninfected *C. parasitica*. Vesicle proliferation of viral infected strains was maintained over time (up to six days post inoculation) whereas in healthy mycelia the amount of vesicles is very low and decreases during the same time period. A subset of vesicles shown to contain viral dsRNA and proteins reacting to CHV1 helicase and polymerase antibodies appeared to be coated when negatively stained and observed at the electron microscope. Moreover the vesicle fraction of CHV1 infected C. parasitica contain a many fold enriched protein band reacting with anti bovine clathrin heavy chain antibodies in western blot analysis; the same vesicle preparation did not show enrichment in proteins reacting with beta COP antibodies. This finding that clathrin accumulates in hypovirulent strains of *C. parasitica* prompted us to clone the C. parasitica clathrin heavy chain gene (CHC) and the middle component of its adapter complex involved in trans Golgi network protein secretion (mu1-adaptin) and to investigate their role in *C. parasitica*.

85 **Conserved SNAREs in *Neurospora crassa* may regulate exocytosis and intracellular fusions.** Gagan D. Gupta and I Brent Heath. York University Biology Toronto, Canada

SNAREs are highly conserved proteins known to be essential for vesicle trafficking and fusion in eukaryotes, but little is known about their presence or role in filamentous fungi. We have identified six SNARE genes in the filamentous fungus *Neurospora crassa*, either via library screening or analysis of current Neuropsora sequencing databases. Three of these genes, syn, nsyn and nsec9 show significant homology to SNAREs involved in the last step of exocytosis at the plasma membrane in Saccharomyces. Hence syn, nsyn and nsec9 may be important for the establishment of an exocytotic gradient that is required for the generation of hyphal tips. Two other identified genes, ntlg1 and ntlg2, bear strong homology to yeast endosomal SNAREs, suggesting the conservation of a Golgi-endosomal trafficking pathway in Neurospora. Another Neurospora gene, nsyn8, appears to have no counterpart in in the fully sequenced Saccharomyces, but bears similarity to an uncharacterized SNARE in *Schizosaccharomyces pombe*, which may indicate the presence of an as yet unidentified route in the secretory pathway in fungi.

86 **Control of cell morphogenesis in fission yeast**. David Wiley, Paola Catanuto and Fulvia Verde. University of Miami, Biochemistry, Miami, FL.

Fission yeast *Schizosaccharomyces pombe* is an excellent model system for studies of cell morphogenesis because it grows in a polarized fashion with a well-defined cylindrical shape. Moreover, polarized cell growth is tightly regulated during the cell cycle. We have previously identified 19 fission yeast genes important for various aspects of cell morphogenesis, and classified them according to their functions during the cell cycle (Verde et al., 1995). One of these, orb6, is required for maintenance of cell polarity, for polarized localization of the actin cytoskeleton, and for its reorganization during the cell cycle (Verde et al, 1998). Orb6 encodes a protein kinase related to human, C.elegans, and *Drosophila* Ndr kinases, *Saccharomyces cerevisiae* CBK1, *Ustilago maydis* Ukc1 and *Neurospora crassa* Cot1. These kinases are related to mammalian Rho-kinase but lack the consensus Rho-binding motifs. Like Orb6, Cot1, CBK1, Ukc1 and Ndr have been shown to be required for the regulation of cell morphology. In fission yeast, disruption of the microtubule cytoskeleton induces cell distortion and cell branching, suggesting an important role for microtubules in cell polarity. Another gene, called *tea1*, encodes a protein which localizes to the cell tips in a microtubule-dependent fashion and is thought to function as a molecular marker for the correct placement of the growth sites (Verde et al. 1995; Mata and Nurse, 1997). In order to investigate the role of Orb6 in the control of cell morphology and to explore the mechanism of Tea1-dependent polarity control, we have conducted genetic screens to identify proteins that interact with Orb6 and Tea1. The results of the characterization of these molecules will be presented.

87 **Control of polarity in the filamentous fungus *Ashbya gossypii.*** Philipp Knechtle, Jürgen Wendland and Peter Philippsen. Applied Microbiology, Biozentrum, University of Basel, Switzerland

We used Alexa-Phalloidin staining to localize the actin cytoskeleton in the filamentous fungus *Ashbya gossypii*. Actin could be observed as patches, cables and as actin rings. Patches localize over the whole cell cortex and polarize at the tips. Patches seem to be interconnected by actin cables. Actin rings localize within the hypha most likely to or at sites of septum formation. Polarized cortical patches could also be observed as a double ring structure

close to the neck between germ bubble and germ tube or within hyphae. The gene product of AgBOI seems to be important in the polarization of cortical actin patches because deletion of AgBOI leads to an occasional loss of polarized growth indicated by spherically enlarged tips. The cortical actin in these enlarged tips is delocalized. Polarized growth can be regained in the same axis which is accompanied by a repolarization of cortical actin. To investigate dynamics of polarized growth we identified a polarity marker by sequence similarity to *Saccharomyces cerevisiae* Spa2p. In *Saccharomyces cerevisiae* ScSpa2p localizes to sites of polarized growth. AgSpa2p shows significant homology to ScSpa2p in its N- and C-terminal part. The internal domain in AgSpa2p is about 2200aa whereas in ScSpa2p it is only 700 aa in length. Both internal domains carry repetitive sequence towards their end but no homology could be observed within the whole internal domains. The genomic copy of AgSPA2 was labelled with GFP at its C-terminus. AgSpa2p-GFP localized to the tips of hyphae. Occasionally an accumulation of AgSpa2p-GFP could be observed as a double ring structure within hyphae. Using video time lapse microscopy we could show that during the development of a young mycelium AgSpa2p-GFP permanently localized to the growing tips. Upon lateral branch formation first an accumulation of AgSpa2p-GFP at the cortex and then continued localization at the tip of the emerging branch can be observed. A similar localization pattern is seen using a genomic GFP fusion to the F-actin binding protein AgCAP1.

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88 **Evolution and functional analysis of mating-type genes (*MAT*) in sexual (*Cochliobolus*) and asexual (*Bipolaris*) fungi**. Saenz, G. S., Berbee*, M. L., and G. Turgeon. Department of Plant Pathology, Cornell University, Ithaca, NY 14853 *Department of Botany, University of British Columbia, Vancouver BC V6T 2C9

In heterothallic fungi, mating-type genes (*MAT*) determine whether a fungal individual can interact favorably with a compatible individual and thus begin the process that leads to sexual reproduction. Asexual fungi either lack the ability to undergo sexual reproduction, or sexual reproduction is cryptic. Since mating-type genes have been identified in asexual species, we can assess the mating potential of a presumably asexual fungus by direct examination of *MAT*. *MAT* genes of sexual *Cochliobolus heterostrophus* are well characterized and provide a basis for comparison with *MAT* genes from closely related asexual fungi, *Bipolaris sacchari* and *B. sorghicola*. We are taking a two-step approach towards determining whether *Bipolaris* spp. have the potential to undergo sexual reproduction: (1) We are sequencing both *MAT1-1* & *MAT1-2* and their flanking regions in these asexual fungi to detect accumulating mutations with respect to *C. heterostrophus MAT*. The accumulation of mutations may result in the loss of *MAT* gene function. (2) We will test function of the *MAT* genes from these asexual species by expressing them in a *MAT* deletion strain of sexual *C. heterostrophus*. If *Bipolaris MAT* genes are able to function in *Cochliobolus*, then this is evidence that *Bipolaris MAT* either maintains the ability to outcross or that the *MAT* genes are selectively maintained for other cellular functions.

89 **Organization and polymorphism of mating-type genes from the bipolar mushroom *Coprinus disseminatus.*** James, Timothy[1], Kues, Ursula[2], and Vilgalys, Rytas[1]. [1]Department of Biology, Duke University, Durham, NC, USA 27708 [2]Institute of Microbiology, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland

The inky-cap mushrooms in the genus *Coprinus* display an extreme diversity of mating systems ranging from completely non-outcrossing to elaborate genetic architectures designed to increase outbreeding efficiencies to nearly 100%, e.g. *C. cinereus*. While the majority of *Coprinus* species have heterothallic mating systems governed by two

unlinked mating-type loci (i.e., tetrapolar mating systems), evolutionary reversions to a mating system controlled by a single locus have occurred (i.e., bipolar mating systems). In order to understand the genetic basis for changes between tetrapolar and bipolar mating determination in mushroom fungi, we are investigating the mating genes of the bipolar mushroom *Coprinus disseminatus*. Using a positional cloning method we have isolated *C. disseminatus* genes homologous to the *A* mating-type homeodomain-encoding genes of *C. cinereus*. Moreover, these genes segregate with mating-type as determined by pairing studies. A chromosome walk will be used to assess whether genes homologous to the *C. cinereus B* mating-type are physically linked to the *A* factor genes in *C. disseminatus*.

90 **Phylogenetic relationships of a new species of Cylindrocladium that causes a blight disease on Buxus spp. with similar taxa, based on morphology and DNA sequences of internal transcribed spacers and beta-tubulin.** Beatrice Henricot. The Royal Horticultural Society, Plant Pathology, Wisley, Surrey, UK

A severe blight disease of *Buxus* spp. was observed in the mid-90s in the UK and since 1998 has spread throughout the whole country. Cases in France, Italy, Belgium and Holland have also been reported. Diseased plants showed dark brown spots on the leaves, black streaks on the stems and severe defoliation. A species of *Cylindrocladium* was consistently isolated from diseased samples and inoculation assays confirmed it as the causal agent of the disease. The morphological description as well as the sequencing of the ITS spacers and the beta-tubulin gene showed that this fungus is a new *Cylindrocladium* species. This species was found to be the same as the one isolated from box plants in New Zealand and initially identified as *Cylindrocladium spathulatum*. The aim of this present study was to use these sequences to infer the phylogeny of this new species and other described *Cylindrocladium* species. An AFLP fingerprint technique was used to try to resolve genetic differences between isolates collected in different geographical locations in the UK and New Zealand. The origin of this new disease will be discussed.

91 **Discordant gene genealogies and the evolution of the trichothecene gene cluster in Fusarium.** Todd J. Ward[1], H. Corby Kistler[2], Joe Bielawski[3], Eileen Sullivan[1], and Kerry O'Donnell[1]. [1]Microbial Properties Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois, USA [2]Cereal Disease Laboratory, Agricultural Research Service, U.S. Department of Agriculture, St. Paul, Minnesota, USA [3]University College, London, Department of Biology, London, England

During the last decade, fusarium head blight, or scab, reached epidemic proportions in the United States, resulting in over 2.6 billion dollars in losses to U.S. agriculture. *Fusarium graminearum* and closely related fungi are the causative agents of scab, and produce trichothecene mycotoxins that act as virulence factors on some hosts, and pose a serious threat to animal health and food safety. In order to investigate the evolution of trichothecene chemotypes within the genus *Fusarium*, and to provide an evolutionary framework for understanding the role of trichothecene mycotoxins in pathogenesis, a 19kb region of the trichothecene gene cluster (including 8 trichothecene genes) was sequenced in *F. graminearum* strains selected to represent the global genetic diversity of this pathogen. Comparisons of trichothecene gene-genealogies with each other and with those derived from genes outside the cluster, in conjunction with an examination of molecular evolutionary patterns, indicated that the species phylogeny is not representative of the evolutionary history of the trichothecene gene cluster. An evolutionary model incorporating multiple population level processes is discussed.

92 **Multiple origins of serotype AD strains in the human pathogenic fungus *Cryptococcus neoformans.*** Jianping Xu[1], Rytas Vilgalys[2], Guizhen Luo[3], Mary Brandt[4], and Thomas G. Mitchell[3]. [1]Dept. of Biology, McMaster University, Canada; [2]Dept. of Biology, [3]Dept. of Microbiology, Duke University, USA, and [4]Mycotic Diseases Division, CDC, Atlanta, USA

Cryptococcus neoformans is an important pathogenic basidiomycetous yeast of humans and other mammals throughout the world. Using commercial monoclonal antibodies to capsular epitopes, strains of *C. neoformans* manifest five distinct serotypes -- A, B, C, D, and AD. To understand the evolution of *C. neoformans*, and in particular, the origin(s) of serotype AD, we investigated 48 strains representing all five serotypes, including 17 different strains of serotype AD. For each of these strains, fragments of two genes (Laccase and Ura5) were sequenced and compared. All strains of serotypes A, B, C, and D, as well as three strains of serotype AD, had only one allele within each of the two loci. However, 14 of the serotype AD strains displayed two different alleles at each locus. Analysis of the sequences of each allele identified significant heterogeneity among the 17 serotype AD strains. Furthermore, significant sequence differences were observed between the two alleles within a locus in each

of the14 serotype AD strains. Phylogenetic analysis revealed that one allele clustered with strains of serotype A and the other with strains of serotype D. The results suggest that serotype AD strains have multiple origins and support the hypothesis that 14 of the17 serotype AD strains originated from recent hybridizations between strains of serotype A and serotype D.

93 **Mating-type gene organisation and field distribution in Discomycete Tapesia species**. Paul S Dyer[1], Greg Douhan[2] and Tim D Murray[2]. [1]School of Life and Environmental Sciences, University of Nottingham, Nottingham UK. [2] Washington State University, Pullman, WA USA.

Mating-type sequences have been cloned from the discomycete plant pathogen *Tapesia yallundae*. Two highly dissimilar DNA idiomorph regions, sizes 3.9 and 3.3 kb were detected in *MAT-1* and *MAT-2* isolates respectively, flanked by regions of nearly identical DNA sequence. Analysis of idiomorph sequences revealed the presence of two putative mating-type genes in the *MAT- 1* idiomorph with conserved alpha-1 and high-mobility group (HMG) domains. An open reading frame for a putative metallothionein-like protein was also present, although this exhibited low homology. A single HMG domain mating-type gene was detected in the *MAT-2* idiomorph. Knowledge of the mating-type sequences was used to devise a multiprimer PCR test for determination of mating-type of isolates of *T. yallundae*. This test employed three primers: a ææcommonÆÆ primer annealing to the idiomorph flanking region of both mating-types, and two ææspecificÆÆ primers annealing to sequences present in either the *MAT-1* or *MAT-2* idiomorphs. Locating the specific primers in different positions relative to the common primer yielded different size 811 bp or 417 bp products characteristic of *MAT-1* or *MAT-2* isolates respectively. The test was used to successfully determine the mating-type of 54 isolates of *T. yallundae*. It was also used successfully to determine the mating-type of isolates of the closely related eyespot pathogen *T. acuformis*. An investigation was made of the distribution of mating-types of *T. acuformis* at field sites worldwide in an attempt to determine possible reasons for the rare occurrence of the sexual cycle of *T. acuformis* compared to that of *T. yallundae*.

94 **A multilocus molecular marker system for studying population subdivision in the rice blast fungus, *Magnaporthe grisea.*** Brett C. Couch and Linda M. Kohn. Botany Department, University of Toronto, Mississauga, Ontario, Canada

The fungus, *Magnaporthe grisea*, is the causal agent of rice blast and gray leaf spot of grasses. It is one of the most important pathogens of rice due to its widespread occurrence and potential for serious crop losses when conditions are conducive to disease development. *M. grisea* comprises a number of host specific populations, based on studies utilizing DNA fingerprinting, RFLPs, and DNA sequence polymorphisms in the ITS region. As well, populations on rice are predominantly clonal based upon DNA fingerprinting studies. However, the possibility for sexual reproduction and recombination exists in populations on other grass hosts. These observations raise two interesting questions. First, how did rice -infecting populations originate? Second, are rice-infecting populations genetically isolated or are migration and gene flow occurring between rice-infecting populations and populations on other hosts? I have developed a molecular marker system suitable for addressing these questions. Fifteen polymorphic DNA genomic regions were identified by direct sequencing of these regions from a set of twenty-one reference isolates of *M. grisea* from rice and other grass hosts. Genomic regions were amplified for sequencing using previously published PCR primers or PCR primers designed from *M. grisea* sequences accessioned in Genbank and from the Clemson University *M. grisea* Genome Sequencing Project.

95 **Identification of a novel invertase in the yellow ecotype of *Neurospora intermedia.*** Alka Pandit and A. J. Griffiths. Department of Botany, University of British Columbia, 6270 University Boulevard, Vancouver, B. C., Canada, V6T 1Z4.

The orange ecotype of *N. intermedia* is found on burnt plant material rich in sugar (like sugar cane), whereas, the yellow ecotype is commonly found on corn cobs. The absence of yellow stains from burnt substrates is striking. Both the ecotypes have similar requirements for growth under laboratory conditions, however, in nature they seem to have substrate preference. The reasons for this substrate preference are the focus of our investigation. Towards this goal morphological and cytological differences between the two ecotypes have been studied. The growth rates, conidial size and nuclear number per conidium have been compared. Both the ecotypes have similar growth rates but show differences in conidial size and nuclear number per conidium. The conidia of yellow ecotype are larger (approx. 1.6 times) than the orange ecotype and the number of nuclei in the conidia of yellow ecotype are about 3

times more than in the orange ecotype. The importance of these differences in adaptation to a particular substrate is unclear. As invertase may play a crucial role in colonisation and adaptation to a sugar rich substrate the invertase activity was assayed in both the ecotypes and the invertase isozyme was studied by gel electrophoresis. The specific activity of the intracellular invertase is more than 6 fold higher in the orange ecotype whereas the specific activity of the extracellular invertase is about 2 fold higher. Interestingly, the enzyme shows polymorphism that is consistent between the two ecotypes. The orange ecotype shows three bands for extracellular as well as the intracellular invertase. The invertase in yellow ecotype differ in the electrophoretic mobility and shows only two bands in extracellular invertase. The differences in the extracellular invertase tempt to suggest an adaptive role in nature. In order to characterise the invertase enzyme and the genes encoding it efforts are being made to amplify the invertase gene from both the ecotypes using PCR.

96 **Relationship between genetic polymorphism and pathogenicity in *Paracoccidioides brasiliensis*.** Flavia V. Morais[1], Kátia C. Cândido [1], Patrícia S. Cisalpino[2], Rosana Puccia[1]. [1]Universidade Federal de São Paulo, São Paulo, Brasil. [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.

Paracoccidioides brasiliensis is the temperature-dependent dimorphic fungus that causes paracoccidiodomycosis (PCM), a human systemic mycosis prevalent in Latin America. The major fungal antigen is the gp43, whose gene is located in a 1,329-bp DNA fragment including two exons, a 78-bp intron, and a leader peptide coding region of 105 bp. Here we describe polymorphism in the gp43 precursor gene after sequencing two PCR fragments from 17 *P. brasiliensis* isolates. The most polymorphic sequences showed 14 - 15 informative substitution sites compared with a consensus sequence and were phylogenetically distant from the others (with 1 - 4 informative sites). They encoded basic gp43 isoforms, generally neutral among the other isolates, and the three isolates in this group were from patients with pulmonary PCM. The biggest clade in a phylogenetic tree included the sequences of isolates from both lymphatic and pulmonary PCM. Preliminary data suggests that these samples are less pathogenic in mice infected i.p with 106 yeast forms, since the number of colony forming units in the spleen and liver was reduced when compared with that of mice infected with four other isolates. The gp43 promotor region (325 bp) showed little polymorphism among the 17 isolates analyzed (1 - 3 informative sites), and none was within transcription motifs. The *P. brasiliensis* isolates were grouped similarly according to the promotor and the gp43 precursor sequences. Sequencing data of the ribosomal ITS 1 and 2 regions of these isolates are being processed and will be compared. Supported by Fapesp, Pronex and CNPq

97 **Phylogenetic characterization of two new isolates of *Histoplasma capsulatum* based on ITS and ETS region sequences from AIDS patients in Japan.** Miki Tamura[1], Takao Kasuga[2], Kayo Watanabe[1], Masakazu Katu[1], Yuzuru Mikami[1], and Kazuko Nishimura[1]. [1]Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chuo-ku, Chiba (260-8673), Japan [2] Roche Molecular Systems, 1145 Atlantic Ave, Alameda, CA 94501, USA

Histoplasma capsulatum is distributed worldwide and causes deep- mycosis in humans. In Asia, the number of cases of the disease histoplasmosis is increasing, however, very little is known about the population structure and pathogenicity of the pathogen. Recently we obtained two clinical isolates in Japan; one from Thai (IFM 49109), and other from Chinese (IFM 49110) AIDS patients. Random amplified polymorphic DNA (RAPD) analysis of both *H. capsulatum* strains showed that they have similar RAPD band patterns to the reference Asian strains, but their patterns were clearly different from those of a North American type 2 reference strain. In this research, we used DNA polymorphisms at the external transcribed spacer region (ETS) and internal transcribed spacer region (ITS) to study the population structure of *H. capsulatum* and to infer the origin of the two Japanese isolates. An unrooted dendrogram constructed from DNA sequences of internal transcribed spacer (ITS) of 27 geographically diverse *H. capsulatum* isolates representing two varieties, *capsulatum* and *duboisii* showed that the isolates could be classified into six clades; Asia type, South America types A and B, North America types 1 and 2, and *H. duboisii* type. The two Japanese isolates IFM 49109 and IFM 49110 were genetically close to North America type 1 group, but distinct. We judged that the two Japanese isolates were unique and thus created a new group the East Asia type. We also analyzed a part of the ETS region. ETS fragment was found to evolve faster and more informative than ITS regions. Dendrogram constructed from ETS sequences also showed that the two Japanese isolates were genetically unique among geographically diverse *H. capsulatum* isolates. DNA sequence analyses of ITS and ETS revealed the geographically differentiated population structure of *H. capsulatum.* Such information is essential to understand the epidemiology and evolution of the clinically important fungal pathogen.

98 **Genetical and physiological diversity of *Cladosporium* spp. sympatrically colonising common reed (*Phragmites australis*).** Stefan G.R. Wirsel[1], Christiane Runge-Froboese [1], Dag G. Ahren[2], Eric Kemen [1], Richard P. Oliver[3] and Kurt W. Mendgen[1]. [1]Lehrstuhl fuer Phytopathologie, Fachbereich fuer Biologie, Universitaet Konstanz, Universitaetstr. 10, D-78434 Konstanz, Germany [2]Microbial Ecology, Ecology Building, Lund University, 22362 Lund, Sweden [3]Australian Centre for Necrotrophic Fungal Pathogens, School of Biological Sciences, Murdoch University, Perth WA 6150, Australia

Species in the fungal genus *Cladosporium* exhibit diverse interactions with plants ranging from facultative biotrophy, to endo- and epiphyty. A collection of 44 isolates with characteristics of *Cladosporium* has been recovered from roots, stems and leaves of surface-sterilised common reed growing at Lake Constance (Germany). Morphological characterisation by high-resolution-cryo-SEM revealed that *Cladosporium* isolates from reed are diverse. Thereby, we distinguished three separate species, i.e. *C. herbarum*, *C. oxysporium* and *Cladosporium* sp. ITS sequence analysis supported these results and, moreover, separated the most common species, *C. oxysporium*, into two subclades. We have established two additional phylogenies in order to verify the placement of reed-associated Cladosporia and to improve the taxonomy of the genus as a whole. One differentiated fungi by their capacities to metabolise 95 different carbon sources using the BIOLOG microtiter system. The deduced phylogeny correlated with the morphological separations. The second phylogeny, based on actin-gene-sequences, showed the same four clades as did the ITS tree, but resulted in a higher resolution indicating putative cryptic species and a high diversity among Cladosporia at the population-species interface. Therefore, by using the phylogenetic species concept we propose that reed is colonised by at least four species of *Cladosporium*. A nested-PCR assay targeting variable sequences within actin introns indicated that these four sympatrically colonise reed. There was no evidence for mutual exclusion on or within the host or specialisation of these fungi for host habitats or organs. However, the incidence of colonisation increased during the season.

99 **Stable polymorphism for the kalilo senescence plasmid in Hawaiian populations of Neurospora.** Alfons Debets, Annelies van Mourik, Anthony J.F. Griffiths*, Rolf F. Hoekstra. Wageningen University, Genetics, Wageningen, The Netherlands. *UBC, Botany, Vancouver, BC, Canada

In this paper we describe the distribution of the kalilo senescence plasmid among 64 isolates of *Neurospora intermedia* and 64 isolates of *N. tetrasperma* from 42 soilsamples taken from Hawaii in 1998. We found that, though the frequency of a 'neutral' Hawaiian plasmid (Han- 2) was significantly lower, the kalilo frequency was similar to that found in 1972 and 1976 and there is still polymorphism for senescence in the *N. intermedia* population. The kalilo plasmid was also present in the *N. tetrasperma* population, and this was strongly correlated with senescence, demonstrating for the first time that kalilo-based senescence is not limited to the *N. intermedia* population of Hawaii. As compared to the *N. intermedia* population, kalilo isolates of *N. tetrasperma* are less frequent and have a longer lifespan. No spatial structuring was observed, there is co-occurrence of kalilo and non-kalilo isolates of both species.

100 **Occurance of gray leaf spot disease of maize in east Africa**. Okori, P., Fahleson. J, and Dixelius, C.. Dept. of Plant Biology, Swedish University of Agricultural Sciences, Box 7080, 750 07 Uppsala, Sweden.

Gray leaf spot disease of maize incited by *Cercospora zeae-maydis* (CZM) is today one of the biggest threats to maize production globally. In East Africa and other parts of the continent, the disease is now ranked second to maize streak gemini virus disease in economic importance. As such, studies of pathogen populations alongside resistance breeding are necessary. A hierarchical survey was used to collect CZM isolates in the major maize producing districts of Uganda, Kenya and Rwanda. Additionally, isolates from Zimbabwe and others previously used in phenetic studies in the United States were included in the study. Monoconidial cultures were then obtained from diseased leaf samples, transferred to potato dextrose broth media and grown for about two weeks. The mycelium was then freeze dried, DNA isolated and polymorphisms studied using AFLP (Amplified Fragment Length Polymorphism). The isolates clustered in two major groups, the African isolates clustered together with the few US group II isolates (NY2, OH8, VA1, GLS3 and GLS5) into one group while the US group I isolates (IN24, IL2, SCOH15 and GLS2) clustered into a small but distinct group. These results were in accordance with a previous investigation (Wang et al. 1998. Phytopathology 88:1269-1275) In general the isolates in their respective groups tended to exhibit growth and other cultural patters as earlier reported. This study support earlier obtained results and shows that group II isolates are prevalent in Eastern Africa while group I isolates was not found.

101 ***Neurospora* in western North America: a model system in the backyard.** David J. Jacobson[1,2], Magdalen M. Barton[2], Jeremy R. Dettman[2], Megan D. Hiltz[2], Amy J. Powell[3], Gregory S. Saenz[3], John W. Taylor[2], N. Louise Glass[2], and Donald O. Natvig[3]. [1]Stanford University, Biological Sciences, Stanford, CA, USA. [2]University of California, Plant and Microbial Biology, Berkeley, CA, USA. [3]University of New Mexico, Biology, Albuquerque, NM, USA.

Species of *Neurospora* have been found mostly in the moist tropics and subtropics. Surprisingly, during the spring and summer of 2000, we observed *Neurospora* in the arid western United States as a primary colonizer of trees and shrubs killed by wildfires, significantly expanding the known geographic range and habitats of the genus. *Neurospora* colonies were observed in 23 forest fire sites in habitats ranging from cottonwood stands along the Rio Grande to mountain forests in New Mexico, the Sierra Nevada and Cascades in California, northeastern Nevada, Idaho, and northwest Montana to the Canadian border. Colonization occurred beneath the bark of diverse deciduous and conifer hosts. The combined 2000 collection includes 314 isolates from 35 to near 49 north latitude and from 750 m to >2400 m altitude. To date, 134 isolates have been identified to species; 130 (97%) are *N. discreta*. Within a site, mating type among individuals is often significantly skewed from a 1:1 ratio. The occurrence of *Neurospora* under these circumstances raises fundamental questions with respect to ecology and population biology: How does *Neurospora* gain access beneath apparently intact tree bark? How is it dispersed or vectored? How and where does it survive for decades between forest fires? What are the reproductive or genetic factors that cause the skewed mating type distribution? The 2000 collection provides a resource to begin addressing these questions.

102 **The HET-s prion, a meiotic drive element causing sporekilling in *Podospora anserina*.** H. Dalstra, F. Debets, K. Swart. Laboratory of Genetics, Wageningen University, The Netherlands.

P. anserina strains of *het-s* genotype exist as two phenotypes,the active [Het-s] and the neutral [Het-s*]. [Het-s] mycelium is vegetative incompatible with mycelium of the *het-S* genotype,whereas [Het-s*] is not.The [Het-s] character is transmitted as an infectious cytoplasmic element. [Het-s] is the prion form of the *het-s* encoded protein. In the classical model of sporekilling, the sporekiller is a segregation distorter, capable of aborting spores containing solely nuclei sensitive to killing. J. Bernet (1965) described a system similar to sporekilling. In a F[Het-s] X M[Het-S] cross at 18 C, up to 50% of the asci contained two normal and two aborted spores. In these asci, the two surviving spores were [Het-s] , whereas offspring from normal asci yielded two [Het-s*] and two [Het-S] spores. This system of sporekilling bears resemblance to the prion directed *het-s/S* vegetative incompatibility system. However in an encounter between [Het-s] and [Het-S] hyphae both sides are equal in hierarchy whereas during sexual crossing the difference in hierarchy is clear, the maternal mycelium being the only cytoplasm donor. In a F[Het-s]XM[Het-S] cross, the *Het-S* nuclei are possibly confronted with a prion permeated environment, potentially leading to an incompatibility reaction and subsequent abortion of young HET-S producing spores. The HET-s prion would act as a meiotic drive element in such a system. The work described in the poster focusses on the role the HET-s prion plays in the Het-sxHet-S type of sporekilling, as it emerges during sexual reproduction in *P. anserina.*

103 **Quantitating the rate of concerted evolution in the ribosomal RNA multigene family**. Austen R.D. Ganley[1], [2]Fred Dietrich and [1]Rytas Vilgalys. [1]Department of Biology and [2]Department of Genetics, Duke University, Durham, NC27708

Concerted evolution describes the unusual evolutionary patterns of repetitive DNA elements whereby the repeats evolve together within a genome. These patterns arise through genomic mechanisms that maintain individual repeats with a consistent sequence, a process known as homogenization. Empirical determination of the homogenization mechanisms has remained elusive, primarily because an experimental system to test mechanisms is lacking. Similarly, the rate of homogenization is unknown and has been assumed to occur over evolutionary timescales. However recent work in fungi and other systems suggests that homogenization may occur relatively rapidly. We are investigating these issues using two approaches. In the first, *Saccharyomyces cerevisiae* is used as a model microevolutionary system to quantitate the rate of homogenization. We have introduced a small neutral change in the ribosomal RNA (rDNA) intergenic spacer, and are monitoring the spread of this "mutant" unit throughout the repeats in an array using a combination of quantitative PCR and pulsed field gels. Determination of a stable "normal" homogenization rate would allow us to use a genetic approach to test homogenization mechanisms. The second approach assesses the level of sequence diversity within an rDNA array using genomic sequencing data from several fungi (e.g. *Ashbya, Cryptococcus, Candida*). Sequence diversity is a product of the homogenization vs

mutation rates, and so provides an indirect estimate of homogenization rate. However it may also be influenced by life cycle mode (e.g. sexual vs asexual reproduction). These experimental systems will aid interpretation of rDNA variability in fungi by providing insights into the dynamics of homogenization on a molecular level.

104 **Analysis of genetic variation in *Peronospora tabacina*using RFLPs.** Serenella Sukno and Mark Farman. Dept. of Plant Pathology, University of Kentucky. S-305 Agricultural Sciences Center, North, Lexington, KY 40546-0091.

Peronospora tabacina Adam is the causal agent of blue mold of tobacco and belongs to the Oomycetes, a diverse group of fungus-like organisms that cause a wide range of destructive and economically important diseases on plants. The inability to identify and track specific *P. tabacina* populations hamper efforts to control blue mold. Such information is vital to the successful implementation of durable disease management strategies in US. To examine the genetics and population biology of this obligate biotrophic parasite, three *Pst*I- genomic DNA libraries were constructed from DNA of three isolates that originated from Kentucky, USA. In preparation for a broader population study, 10 strains representing populations from Kentucky, Florida, Texas, Georgia, Pennsylvania, and Connecticut, were selected for an initial survey of RFLPs markers. *Pst*I- and *Dra*I- digested DNA were hybridized to 10 probes. Preliminary analysis indicates that there is a low level of genetic variation among the US populations. Two polymorphic probes were identified and seven different haplotypes could be differentiated among 10 isolates. Experiments are currently under way to evaluate the somatic stability of single spore lineages of one isolate and variability among individuals in a population. Results of the studies will be presented.

105 **Ascospore morphology is a poor predictor of the phylogenetic relationships of Neurospora and Gelasinospora.** Jeremy R. Dettman, Fred M. Harbinski, and John W. Taylor. Plant and Microbial Biology, University of California, Berkeley, CA, 94720

The genera Neurospora and Gelasinospora are morphologically comparable except the former produces ascospores with longitudinal elevated ridges (ribs) separated by depressed grooves (veins), and the latter genus produces ascospores with spherical or oval indentations (pits). Within a genus, the patterns of ascospore ornamentation can vary quite significantly between species, which suggests these unifying morphological characteristics may not be homologous. To assess the possibility of multiple independent origins of "ribbed" or "pitted" ascospores, the DNA sequences of four nuclear genes were obtained for 12 Neurospora taxa and four Gelasinospora taxa. Within the genus Neurospora, only three well-supported conclusions could be drawn: 1) the five outbreeding conidiating Neurospora species form a monophyletic group, 2) N. discreta is the most divergent of these five species, and 3) four homothallic Neurospora species form a monophyletic group. Evidently, the Neurospora and Gelasinospora taxa included in this study do not represent two clearly resolved monophyletic sister genera, but instead represent a polyphyletic group of taxa with close phylogenetic relationships and significant morphological similarities. The hypotheses of the monophyly of either pitted or ribbed ascospores could be rejected, which suggested that multiple origins of at least one of the character states were likely. Ascospore morphology, the character that the distinction between the genera Neurospora and Gelasinospora is based upon, was not an accurate predictor of phylogenetic relationships as inferred from the sequence data analyzed in this study.

106 **Genetic variation of *Colletotrichum lindemuthianum* in bean fields**. Raul Rodríguez-Guerra, Maria-Teresa Ramírez-Rueda, Octavio Martínez de la Vega and June Simpson. CINVESTAV, Unidad Irapuato, Apdo. Postal 629, Irapuato, Gto. Mexico.

Colletotrichum lindemuthianum is the causal agent of anthracnose in common bean (*Phaseolus vulgaris*). Analysis of monospore cultures from different bean fields in different regions of Mexico has shown that all such isolates can be distinguished at the genotype level using molecular markers but may often share pathotypes. The high level of variability is surprising since *C. lindemuthianum* is not known to undergo a sexual cycle under field conditions. Two commercial bean fields and one experimental plot were chosen in order to study variability in detail in terms of pathotype, capacity for anastomosis and molecular marker genotype of numerous isolates from each location. In addition one commercial field was sampled and analyzed in two consecutive years. Single spore isolates from a single plant were identical in terms of pathotype and capacity for anastomosis and the majority also had identical genotype patterns. A few isolates showed differences in a few bands. When single spore isolates from different individual plants were analyzed, all were found to have the same pathotype and very closely related or identical

genotype patterns. In one commercial field and the experimental plot, differences could be determined in capacity for anastomosis where distinct groups could be determined in each case. In the commercial field where samples were taken in two consecutive years, in the second year although the capacity for anastomosis remained the same and genotpyes were also very similar, a new pathotypewas observed capable of infecting one extra cultivar of the differential set. We are grateful to CONACyT (K0195B) and SIHGO

107 **Molecular clocks in Plectomycetes and the radiation of *Histoplasma.*** Takao Kasuga[1], Thomas J. White[2] and John W. Taylor[3]. [1]Roche Molecular Systems, Alameda, CA. [2]Applied Biosystems, Foster City, CA. [3]Plant & Microbial Biology, Univ. California, Berkeley, CA.

Owing to the scarcity of paleontological record, which is a prerequisite for calibration of time points, the nucleotide substitution rate in fungi has not been reported except for the small subunit ribosomal RNA gene (SSU rDNA). By using the published DNA substitution rate at the SSU rDNA as a time-standard together with pairwise DNA diversity data between closely related species, we estimated DNA substitution rates of seven independent protein-coding genes and the internal transcribed spacer (ITS) region in plectomycetes. Comparative analyses of multiple species showed that the DNA substitution rate in the class 1 chitin synthase gene was approximately constant across plectomycetous fungi, whereas that of ITS varied almost 10-fold. The estimated substitution rates at synonymous sites in protein coding genes ranged from $2.7x10^{-9}$ to $23.9x10^{-9}$ substitutions per site per year. These values are in the range of synonymous DNA substitution rates for a majority of protein coding genes in plants, animals and bacteria, despite of the enormous differences in body size, cellular organization, generation time and ecology.

A human pathogenic fungus *Histoplasma capsulatum* was believed to harbor three varieties, which showed differences in clinical manifestations and geographical distribution. DNA sequence variation in four independent protein genes revealed that *H. capsulatum* consisted of at least eight independent phylogenetic species. Combining the estimated DNA substitution rates with the phylogeny suggests that the radiation of *Histoplasma* started roughly 5 million years ago in South America.

108 **Sexual recombination and dispersal *of Cryptococcus neoformans* var. *gattii* in the natural environment.** Catriona Halliday and Dee Carter. University of Sydney, Microbiology, Sydney NSW Australia

Cryptococcus neoformans causes cryptococcosis in humans and animals, which is thought to begin by inhaling an infectious propagule from an environmental source. In Australia, *C. neoformans* var. *gattii* is most frequently isolated from Eucalyptus trees, which are thought to be the primary ecological niche of this variety. Understanding the occurrence of the fungus on host trees and its dispersal from these trees are likely to be important for assessing the risk of exposure to infectious propagules. We have therefore investigated the population structure of *C. neoformans* var. *gattii* isolates obtained from a number of host eucalypts within a limited geographical range. We began by assessing the potential for sexual recombination between isolates in this population, as dissemination is likely to occur via sexually produced basidiospores. The two mating types were found to be present in close to the 50:50 ratio expected by sexual outcrossing. However, when the structure of the population was assessed using AFLP loci a clonal pattern emerged. Finally, Canonical Analysis of Variance (CVA) of the AFLP dataset found a distinct division of genotypes according to their host tree, suggesting transmission between trees in this area is limited. We conclude that although the prevalence of *C. neoformans* var. *gattii* in this area is high and the potential for sexual recombination exists, the fungus does not commonly complete its lifecycle in association with the host trees but instead propagates as an asexual yeast that is not readily dispersed.

109 ***Cryptococcus neoformans*: global molecular epidemiology.** Wieland Meyer, Krystyna Maszewska, Mathew Hugnh and Sarah Kidd. Molecular Mycology Laboratory, Centre for Infectious Diseases and Microbiology, The University of Sydney at Westmead Hospital, Westmead, NSW, Australia

C. neoformans is a basidiomycetous yeast with three suggested varieties: var. *grubii* (serotype A), var. *neoformans*, (serotype D) and var. *gattii* (serotypes B and C). Varieties *grubii/neoformans* infect immunocompromised patients while variety *gattii* mainly infects immunocompetent hosts. The global genetic distribution of *C. neoformans* was studied by PCR-fingerprinting with single primers specific to minisatellite or microsatellite DNA. Clinical/environmental isolates obtained from around the world grouped into 8 major molecular types (VNI and

VNII = serotype A, VNIII = serotype A/D, VNIV = serotype D and VGI, VGII, VGIII and VGIV = serotypes B and C). VNI and VGI were the most common genotypes. VGIII was geographically restricted to India/USA and VGIV to India/South Africa. Unique, strain-specific patterns were found for most of the US isolates, indicating a high degree of genetic diversity compared to isolates obtained from other areas in the world. Non-US isolates were highly genetically homogeneous or even clonal. When analysed with GelComparII the strains clustered broadly according to their country of isolation. Some strains were common to different countries. The overall global similarity between strains was 60%. Our findings support a division of *C. neoformans* into three varieties or even three separate species. Isolates obtained from the same patient at different time points and different body sites had identical banding patterns indicating a single source of infection. Regional profiles of eucalypt-derived and clinical isolates were concordant, supporting an epidemiological association between these trees and human infection. An automation of the methodology is currently underway.

110 **Mitochondrial plasmids in *Cryphonectria parasitica.*** Gobbi, E. and Rekab, D. University of Udine, DBADP, Udine, Italy.

The first plasmid reported in *Cryphonectria parasitica* was a mitochondrial (mt) plasmid pUG1, found in an Italian strain of the plant pathogen. It belongs to the small group of mt circular plasmids sharing no homology with the host DNAs together with plasmids of *Neurospora spp.*, *Pythium spp.* and *Absidia glauca*. These plasmids propagate by using a plasmid encoded DNA polymerase whose features constitute and characterise a distinct subgroup of enzymes and of corresponding mt plasmids. The putative amino acidic sequence of pUG1 shares a high degree of similarity with those of two *Neurospora intermedia* plasmids, Fiji and LaBelle. They are characterised by a specific signature in the motif C typical of the family B DNA polymerases, TTD instead of DTD, that presumably is a typical motif of this subgroup of enzymes. The three plasmids also share a large number of amino acids typical of the protein primed DNA polymerases found in linear mt plasmids, linear bacteriophages and viruses. Finally pUG1, Fiji and LaBelle show a similar size and structure of their genomes confirming their belonging to a distinct group. In the context of the evolutionary and population biology of the pUG1 plasmids, a survey of a world collection of 154 strains of *C. parasitica* was conducted, the isolates were screened by specific PCR and amplified DNAs from some representative strains were sequenced and analysed.

111 **The *scooter* transposons are not unique to *Schizophyllum commune.*** Cynthia L. St. Hilaire, Thomas J. Fowler, and Carlene A. Raper. Department of Microbiology and Molecular Genetics, University of Vermont, Burlington VT 05405 USA.

Scooter transposons are DNA-mediated transposons first identified in the homobasidiomycete *Schizophyllum commune*. Two copies of *scooter* previously characterized, *scooter-1* and *scooter-2*, are 91% identical over their ~ 650 bp. They are nonautonomous elements; an autonomous element has not yet been identified. *Scooter* elements can transpose and their insertions have led to gene disruptions. Depending on the strain of *S. commune* tested, anywhere from 3 to 25 restriction fragments have been identified with a *scooter* probe on genomic Southern blots. We were curious as to whether *scooter* was confined to this one species, or if other mushroom fungi also harbor *scooter* elements. Genomic DNA from several mushroom species was probed with *scooter* at low stringency in a Southern blot analysis. Hybridization with *scooter* was evident in several of other mushroom fungi, including *Agaricus bisporus, Pleurotus ostreatus,* and *Lentinus edodes,* but the hybridization signal is much weaker for these other fungi than for *S. commune.* Different varieties of *A. bisporus*, the button and Portobella varieties, have similar hybridization patterns to suggest these *scooter*-related sequences may not transpose in *A. bisporus.* We are attempting to isolate some of these *scooter*-related sequences with PCR to ascertain their relationship to the *S.commune scooter* elements.

112 **AFLP diversity of *Cephalosporium maydis* in Egypt.** A. A. Saleh[1], K. A. Zeller[1], E. M. El-Assiuty[2] and J. F. Leslie[1]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502 [2]Maize Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

We characterized more than 868 isolates of *Cephalosporium maydis* collected from 14 governates in Egypt with AFLP markers. These governates included seven located in lower Egypt and seven located in upper Egypt. The four primer-pair combinations resulted in 25 polymorphic markers from total of 68. UPGMA clustering analysis results in four groups (lineages) that are not uniformly distributed throughout the country. Lineage four was not recovered

from any of the seven upper Egypt governates whereas the other three lineages were found in both upper and lower Egypt. Lineage four was the most divergent group. In some locations, one lineage dominated (up to 98%) and some fields were colonized only by isolates that belonged to the same clone. Differences in climate or maize varieties planted might be sufficient to explain the unusual distribution of lineage four.

113 **Cloning and analysis of the mating type genes from the barley pathogen *Septoria passerinii***. Stephen B. Goodwin[1], Cees Waalwijk[2], Gerrit H. J. Kema [2] and Jessica R. Cavaletto[1]. [1] USDA-ARS, Department of Botany and Plant Pathology, 1155 Lilly Hall, Purdue University, West Lafayette, IN 47907-1155; [2] Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Septoria passerinii causes speckled leaf blotch of barley. No teleomorph has been found and the pathogen is assumed to reproduce through asexual pycnidiospores. Recent phylogenetic analyses have shown that *S. passerinii* is closely related to the wheat pathogen *Mycosphaerella graminicola*, a species with regular sexual reproduction. Analyses of genetic variation within populations of *S. passerinii* revealed a high level of genotypic diversity, suggesting the possibility of sexual reproduction in nature. To test the hypothesis that *S. passerinii* is capable of sexual reproduction, mating type clones from *M. graminicola* were used as probes in Southern analyses. A 4 kb band was present in some isolates of *S. passerinii* when probed with a clone containing the HMG box from *M. graminicola*. This 4176 bp band contained a complete HMG box-like idiomorph of 2899 bp from *S. passerinii*, including sequence at both flanking regions. An open reading frame (ORF) of 101 amino acids had high similarity to the HMG box region of *M. graminicola* and other fungi. PCR primers were designed to amplify the other mating type idiomorph from isolates that did not hybridize to the HMG box probe. Sequencing of a 3637 bp PCR product revealed an alpha protein-containing idiomorph of 3050 bp. This clone contained an ORF of 345 amino acids with high similarity to alpha mating-type proteins from other fungi. Primers for multiplex PCR were designed to test the mating types from field isolates. This technique revealed that both mating types were present in the same fields in Minnesota and North Dakota. Therefore, this pathogen may have the potential for sexual reproduction in nature, which could explain the high levels of genotypic diversity observed in barley fields in the north central U.S.

114 **Evidence for domestication of the fungal symbionts of leafcutter ants**. Stephen A. Rehner. Insect Biocontrol Laboratory, ARS, USDA. Beltsville, Maryland, USA.

Whether the fungi cultivated by leafcutter ants are truly domesticated has posed an enigma for over 100 years. Analyses of genetic variation among leafcutter cultivars at local and geographic scales were conducted to see if their genetic structure departed from a null expectation of a recombinatorial interbreeding population structure. AFLP variation among cultivars from central Panama revealed a hierarchical genetic structure consistent with the traditional view that the fungi are clonal. However, mycelial incompatibility between pairs of genetically different cultivars provide evidence that these fungi both retain and exercise the ability for sexual reproduction. Phylogeographic analysis of cultivars demonstrated that alleles of different loci are concordantly partitioned among geographically structured. These data support the conclusion that the leafcutter fungi comprise a series of inbreeding cultivar lines that exist predominantly, if not exclusively, in association with their ant hosts.

115 **Evolutionary relationships of kinesins in fungi.** Conrad L. Schoch, B. Gillian Turgeon, Olen C. Yoder and James R. Aist. Department of Plant Pathology, Cornell University, Ithaca, New York

Kinesins are mechanochemical proteins able to move cargo along microtubules by ATP hydrolysis. Together with dyneins and myosins they are motor proteins, involved in a number of vital cellular processes such as organelle transport, chromosome segregation and cytokinesis. These proteins have been isolated from a wide range of organisms ranging from humans to yeast and their evolutionary relationships compared and analysed in previous studies. Among fungi valuable insights into function have been gained from functional analysis in organisms such as the ascomycetous yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, the filamentous ascomycetes *Aspergillus nidulans*, *Neurospora crassa* and *Nectria haematococca*, as well as the basidiomycete *Ustilago maydis*. A computer generated search with available genomic sequences of the plant pathogenic fungi *Fusarium graminearum*, *Cochliobolus heterostrophus* and *Botrytis cinerea* has indicated that the numbers of putative genes containing kinesin motor domains in each fungus is comparable to those already known from *S. cerevisiae*. A phylogenetic analysis is presented.

116 **The use of molecular phylogenies for estimation of fungal diversity.** Jean-Marc Moncalvo and Rytas Vilgalys. Duke University Department of Biology, Durham NC 27708

Classic estimates of biological diversity use species (or genera) as units of measurement. The development of large-scale molecular phylogenies provides an unique opportunity to estimate biological diversity at the genetic level by using the branching order and branch lengths from phylogenetic trees. Both among-taxa and among-area phylogenetic diversity can be estimated using a "phylogenetic index of diversity" (PD). We will contrast PD with classic estimates of biological diversity in various groups of mushrooms for which extensive geographic sampling and molecular phylogenies are available, including genera *Amanita, Pleurotus, Lentinula,* and *Ganoderma*. There are at least two advantages of PD over traditional measures of estimating biological diversity: 1) PD does not require a priori knowledge of taxonomic circumscription (which is generally subjective in fungi), and 2) PD takes into account genetic distances, therefore emphasizes genetic breadth when estimating diversity.

117 **Molecular population genetics of arbuscular mycorrhizal fungi: Are they clonal or recombining?** Teresa E. Pawlowska and John W. Taylor. University of California, Plant & Microbial Biology, Berkeley, CA 94720, USA.

Arbuscular mycorrhizal fungi (Glomales) form symbioses with the majority of plant species, including many crops. They facilitate plant mineral nutrition and confer tolerance to pathogens, drought, salinity and metal toxicity. Glomales are obligate biotrophs with no evidence of sexual reproduction. The location of genetic variation in their multinucleate hyphae is unknown. It may be in each nucleus (homokaryotic) or among several nuclei (heterokaryotic). Before we can challenge the hypothesis that Glomales are clonal, we need to establish whether they are homo- or heterokaryotic. As a model we are using *Glomus etunicatum*, a ubiquitous species that can be easily sampled from nature and cultured *in vitro*. To obtain a representation of its wild populations, we collected soil samples from Berkeley, CA, and St. Paul, MN. Spores recovered from greenhouse trap cultures were used to initiate single spore cultures of *G. etunicatum* in association with excised Ri T-DNA transformed carrot roots. To search for polymorphic markers in single copy genes in addition to rDNA arrays, and to enable PCR amplification of multiple loci from individual *Glomus* spores, we optimized a strategy for global amplification of spore DNA. Putative genes encoding a DEAD box protein, a catalytic subunit of DNA polymerase alpha, a protein with a putative leucine zipper DNA-binding element, and an ADP- ribosylation factor-like protein were identified as genetic markers. To test for homo- vs. heterokaryosis, we are analyzing progeny spores from single spore cultures for variation at the polymorphic loci. If all the progeny are identical, there would be no evidence for heterokaryosis, and we would be able to proceed to testing of reproductive mode of *Glomus* in nature.

118 **The quest for nucleotide polymorphism in *Aspergillus fumigatus*; implications for a phylogenetic definition of the species.** J.L. Platt[1], D.M. Geiser[2], & J.W. Taylor[1]. [1]University of California, Berkeley, CA USA. [2]The Pennsylvania State University, University Park, PA USA.

Phylogenetic analyses of several human pathogenic fungi have shown that species traditionally defined on the basis of morphology may actually represent more than one genetically isolated species. As a first objective in our effort to understand the reproductive mode and population genetics of *Aspergillus fumigatus,* we are re-examining the species boundary in this asexual pathogen. We conducted a preliminary screening for polymorphic sites in 28 loci of both clinical and environmental isolates. Phylogenetic analyses of nucleotide sequence data from eight loci in exemplar isolates that include two varieties of *A. fumigatus*(var. *acolumnaris* and var. *ellipticus)* were used to infer existence of genetic isolation. While sequences of catalase (*catB)* and polyketide synthase (*pksP)* showed some nucleotide variation within all isolates, the remaining loci (*chsG, alp1, mep,*and *gp55)*exhibited very little nucleotide variation even between varieties. A comparison of the resulting gene genealogies did not result in any patterns that would be consistent with cryptic speciation and is counter to other studies which indicate that cryptic speciation may be relatively common in fungi. We are now pursuing several other potential sources of polymorphic nucleotide sites, including uncharacterized loci from BAC end sequences, intergenic regions, and microsatellite flanking sequences. Phylogenetic analyses of more variable markers will be used to further test the traditional morphology-based species concept of *A. fumigatus.*

119 **Population genetics of *Ustilago maydis* as determined by RFLP and allelic variation and the b mating type locus.** James R. Garton, Georgiana May and Christine E Ramos. University of Minnesota, Plant Biology, St. Paul, MN

We investigated the population genetic structure of *Ustilago maydis*, with particular emphasis on migration levels between geographically distant populations. We examined genetic diversity in this organism by the use of 13 RFLP probes and characterization of allelic diversity at the b mating type locus. We performed analyses on 276 haploid individuals sampled from seven subpopulations. Six of these populations were in the US while one was from Uruguay. Low to moderate genetic variability was observed at the neutral loci in all US populations (pairwise F_{st} ranges from 0.1 to 0.25), while substantial variability was found between US populations and Uruguay (average F_{st} of 0.4). The variability detected at the b-locus was universally low (F_{st} of 0.1). Calculation of migration levels by Slatkin's private allele method revealed moderate gene flow in the US populations (N_m=3), and no gene flow between the US and South America (N_m=0.3). Mantel tests revealed no correlation between genetic and geographic distance for populations in the US (correlation coefficient of -0.006), and moderate correlation between the US and Uruguayan population (correlation coefficient of 0.6). The low level of genetic diversity observed at the mating type locus is the result of frequency dependent selection maintaining a high level of variation at this locus. It is our conclusion that the results from the neutral markers indicate that this fungus demonstrates moderate levels of short range gene flow while virtually no migrants are exchanged over long distances (South America to the US).

120 **Clone size, fine-scale population structure, and phylogenetic species in the ectomycorrhizal false-truffle *Rhizopogon vinicolor* complex.** Annette M. Kretzer[1], Lisa C. Grubisha[2], Randy Molina[3], and Joseph W. Spatafora[1]. [1]Dept. of Botany & Plant Pathology, Oregon State University, Corvallis, OR, USA. [2]Dept. of Plant & Microbial Biology, University of California, Berkeley, CA, USA. [3]US Forest Service, PNW Research Station, Corvallis, OR, USA.

A population genetic study was initiated to study the population dynamics of the ectomycorrhizal false-truffle *Rhizopogon vinicolor*. *R. vinicolor* is host specific with *Pseudotsuga menziesii* and produces hypogeous sporocarps and nonforcibly discharged spores that are primarily dispersed through small mammal mycophagy. *R. vinicolor* was chosen as a model system for studying population biology of truffle-forming ectomycorrhizal fungi for numerous reasons including host specificity, common occurrence in nature, and ease of sampling. This last factor is attributed to the fact that *R. vinicolor* is the only false-truffle reported to produce tuberculate mycorrhizae, which consist of clusters of ectomycorrhizal roottips encased in a peridium. These tuberculate mycorrhizae are relatively easy to sample in nature and are more widely distributed than sporocarps. We developed numerous *R. vinicolor*-specific, single-copy microsatellite markers to address several questions including clone size and distribution, fine scale population fragmentation, and gene flow in the context of isolation by distance and barriers. Our initial results supported that clone size ranged from less than 5 meters to approximately 15 meters in diameter. More exhaustive sampling and analyses revealed that two sympatrically distributed Rhizopogon species produce tuberculate mycorrhizae, which we distinguish here as *R. vinicolor s.s.* and *R. cf. vinicolor*. These results were corroborated by the sampling of sporocarps including type specimens, the lack of shared alleles across microsatellite loci, and through phylogenetic analysis of the ITS rDNA. We will also present preliminary data on fine-scale population structure at the water shed level and relative rates of inbreeding.

121 **Combined genotyping methods for indoor moulds.** James Scott[1] and Wieland Meyer[2]. [1]University of Toronto, Botany, Toronto, ON. Canada. [2]University of Sydney, Westmead Hospital, Westmead NSW Australia.

Penicillium chrysogenum is one of the most common microfungi isolated from indoor environments. Moreover, this species is of considerable industrial importance as the principal producer of the antibiotic, penicillin. This study used two genotyping methods, heteroduplex mobility assay (HMA) and PCR fingerprinting to examine the extent of clonality within this species.

Thirty-eight isolates of *P. chrysogenum* were obtained from broadloom dust representing 27 houses from Wallaceburg, Ontario, Canada and neighboring rural areas. One isolate each of *P. polonicum* and *P. thomii* were used as outgroups. Allelic variability was assessed using HMA in PCR-amplified polymorphic genetic loci including three regions spanning introns in the conserved structural or metabolic genes acetyl-Coenzyme-A synthase (acuA), beta- tubulin (benA) and thioredoxin reductase (trxB), as well as the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA gene. The identity of alleles was confirmed by sequencing. The same panel of isolates was genotyped by PCR fingerprinting using single-primer PCR with the minisatellite-specific core sequence of the wild-type phage M13, and the microsatellite-specific motif $(GACA)_4$. In addition, 2-primer PCR was conducted using the two RAPD primers, 5SOR and MYC1.

HMA revealed three alleles at each locus that assorted as 5 multilocus haplotypes. All alleles showed strong association and no incompatibilities were observed, indicating strict clonality. Phylogenetic analysis of sequences of the combined loci revealed three well-supported clades. Results of PCR fingerprinting similarly identified three primary lineages, however, these methods provided considerably better resolution within these clades. The taxonomic implications of these results will be discussed.

122 **Evolution of spacer regions of nuclear ribosomal multigene family in *Fusarium culmorum*.** P. K. Mishra, R. T. V. Fox and A. Culham. School of Plant Sciences, The University of Reading, Whiteknights, RG6 6AS, U.K.

The genus *Fusarium* contains many agronomically and clinically important species. *Fusarium culmorum* is of particular importance due to its significance not only in plant pathology but also in mycotoxicology. In this study, we analyze the evolutionary dynamics of the spacer regions of multigene family of nuclear ribosomal DNA. The sequence data analyzed deruve from the internal transcribed spacer (ITS) and intergenic spacer (IGS) regions of seventy five strains of *Fusarium culmorum* representing different hosts and geographical origins. Our extensive molecular analysis of sequence data reveals a contrasting pattern of evolution in ITS and IGS regions. Multiple examples of apparent gene duplication, substitutions and indel events were observed in IGS region. In contrast, it seems that ITS region is completely homogenized among the *Fusarium culmorum* strains exhibiting only 0.35% sequence divergence. Thus, it seems apparent that a different mode of concerted evolution is operating in the spacer regions of nuclear ribosomal DNA in *Fusarium culmorum*. The possible models of homogenization have been discussed.

123 **Analysis of a eukaryotic microbial mat community across environmental gradients in a thermal, acidic stream.** Kathy B. Sheehan, Michael J. Ferris, and Joan M. Henson. Department of Microbiology and the Thermal Biology Institute, Montana State University, Bozeman, MT

Nymph Creek in Yellowstone National Park is a natural laboratory for understanding eukaryotic microbial genetic diversity, ecophysiology, and behavior. The thermal(50ºC), acidic(pH 2.7) water creates stable environmental gradients in temperature, pH, and light over which changes in microbial populations are being monitored using microscopic methods and rRNA sequencing during diurnal and seasonal periods. The mat is primarily composed of the red alga, *Cyanidium caldarium*, the most thermophilic alga known, and the thermophilic, filamentous fungus, *Dactylaria constricta* var. *gallopava*. The distributions of fungal and algal 18S rRNA genotypes provide information on how genotypes correlate with ecological niches. Of particular interest is the interaction between *C. caldarium* and *D. constricta*, which could represent a primitive symbiotic ancestor to modern-day lichens. In addition, we are studying whether the alga provides nutrients for the fungus, whether the fungus parasitizes the alga, whether fungal hyphae provide a matrix that anchors the unicellular alga to form a mat and whether fungal melanins protect the alga from the harmful effects of intense light at high elevations. This project is supported by a National Science Foundation Microbial Observatory grant and by the Thermal Biology Institute at Montana State University.

124 **Human disturbance fosters hybridization in the fungal tree pathogen Heterobasidion.** M. Garbelotto, W. Otrosina, and I. Chapela. University of California, ESPM-Ecosysytem Sciences, Berkeley, CA, USA

Hybrids of two host-specific taxa in the pathogenic fungal genus *Heterobasidion* were less virulent than parental strains on either of the adaptive hosts in greenhouse experiments. However, we did not find a hybrid disadvantage on stumps and on the dual-host Sitka spruce, representing alternate non-selective infection courts. In California, non-selective courts have been massively produced only in the recent past, due to logging and fire-control practices. We provide evidence that these two management practices have lead to conditions conducive to hybridization and interspecifiec gene flow. Based on discordant genealogies we show that interspecific gene flow has also occurred in the past between the two North American taxa of the genus *Heterobasidion* 39 Using a Shot-Gun Genomic Microarray to Probe Pathogenesis in *Histoplasma capsulatum.* Margareta Andersson, Adam Bahrami, M. Paige Nittler, and Anita Sil The goal of our work is to identify regulators of pathogenesis in the dimorphic fungus *Histoplasma capsulatum,* a primary pathogen that causes severe disease in immunocompromised patients. *H. capsulatum* grows in a mycelial form in soil. Conidia or hyphal fragments are inhaled by the host; once inside the host, the cells undergo a morphogenetic switch and grow as a budding yeast form which parasitizes macrophages. The molecular regulators of this dimorphic switch, which is thought to be essential for establishment of infection, are unknown. Similarly, how *H. capsulatum* is able to escape killing by macrophages and colonize the

phagolysosome, an intracellular niche that is normally hostile to microbes, is a mystery. We, in collaboration with Lena Hwang and Jasper Rine at UCBerkeley, have designed a powerful new tool that can be used to expeditiously identify key regulatory proteins in the life cycle of this dimorphic pathogen. We have built a 9600- element H. capsulatum DNA microarray and have used it successfully to identify new yeast-specific and mycelial-specific genes whose differential expression has been confirmed by Northern analysis. We are now poised to use genomics as a high through-put method to dissect key steps in the infectious process such as colonization of macrophages. We will present preliminary data pertinent to the gene expression profile of *H. capsulatum* that has colonized macrophages.

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Genomics and Proteomics

125 **Physical mapping and functional analysis of chromosome IV pericentric region in *Aspergillus nidulans*.** Alexei Aleksenko and Michael Lynge Nielsen. BioCentrum, Technical University of Denmark, Bygn. 223, Lyngby 2800 Denmark

Chromosome walk between genes *methG, uvsB, hisA, gdhB* and *frA* of *A. nidulans* produced two continuous segments of a physical map of chromosome IV covering 250kb and 160 kb. Integration of selectable markers into the chromosome targeted by unique cosmid fragments made it possible to establish that the two fragments flank the centromere, with the order of markers *methG-uvsB-CEN-hisA-gdhB-frA*. The gap in the physical map over the centromere itself was estimated to be between 70 kb and 250 kb. It was shown that the frequency of meiotic recombination in the vicinity of the centromere is strongly reduced. Pericentric fragnets contain highly repetitive AT-rich DNA and degenerated retrotransposons. Centromere-proximal DNA fragments were subcloned in circular and linear vectors, and their behavior in transformation was studied.

126 **Heterologous transposition in the phytopathogenic fungus *Ustilago maydis* using the *Caenorhabditis elegans* element Tc1.** Oliver Ladendorf and Joerg Kaemper. Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., D-35043 Marburg, Germany

Development of molecular techniques for phytopathogenic fungi aimes at the identification of genes whose products are essential for a successful infection of the host plant. Initial approaches have relied on isolating genes and generating knockouts by transformation-mediated insertional mutagenesis. This method presents several drawbacks like the formation of tandem integrations or the creation of DNA rearrangements. As an alternative strategy we have now exploited the versatility of transposons for the generation of random mutations in the phytopathogenic basidiomycete *Ustilago maydis.* For this purpose we have taken advantage of the Tc1 element from *Caenorhabditis elegans,* known to transpose independently of host-specific factors in evolutionary distant organisms. We have constructed an *U. maydis* strain constitutively expressing the Tc1 transposase and harbouring the Tc1 transposon with a hygromycin resistance gene on a free replicating plasmid. The use of the *nar1* gene (coding for nitrate reductase) as a marker allowed both the selection (on nitrate medium) and counterselection (on chlorate medium) of the transposon-donor-plasmid. Upon curing of the plasmid, hygromycin resistant colonies, indicative for integration events of Tc1 into the genome, were identified. Via inverse PCR it was possible to determine the positions of integrated Tc1 elements. The repetitive ends of the transposon were not altered and the integration was accompanied with a duplication of the dinucleotide TA at the target site, consistent with data for transposition events in *C.*

elegans. The experiments demonstrate for the first time the transposition of a heterologous transposon in *U. maydis* and underline the potential of Tc1 as a molecular tool.

127 **Identification of major membrane glycosylphosphatidylinositol-anchored proteins in *Aspergillus fumigatus.*** Jean-Michel Bruneau[1] , Thierry Magnin[2] , Eric Tagat[1] , Raymond Legrand[3] , Muriel Bernard[2] , Michel Diaquin[2] , Claude Fudali[3] , and Jean-Paul Latge[2]. [1] Infectious Disease Group, Biochemistry Department, and [3] Core Research Functions, Biophysics Department, Aventis Pharma, 93235 Romainville, France and [2] Unit des Aspergillus, Institut Pasteur, 75724 Paris, France

Previous studies (Mouyna I., Fontaine T., Vai M., Monod M., Fonzi W.A., Diaquin M., Popolo L., Hartland R.P., and Latg J.-P. (2000) J. Biol. Chem. 275: 14882- 14889) have shown that glycosylphosphatidylinositol anchored proteins play an important role in fungal cell wall biosynthesis. GPI-anchored proteins of *Aspergillus fumigatus* have been purified and separated by 2-dimensional electrophoresis. They were characterized by their peptide mass fingerprint through MALDI-TOF mass spectrometry and by internal amino-acid sequences obtained by Edman sequencing, nano-ES-MS/MS or Q-TOF-MS/MS. Most identified proteins were homologous to putative GPI-anchored proteins present in other fungi. One protein only displayed amino-acid sequences not found in sequence databases. Several of the GPI-anchored proteins identified in *A. fumigatus* were orthologs of genes (CRH1, CRH2, ECM33, GAS1) known to play a role in yeast cell wall biogenesis. Furthermore, a comparative study performed with chitin synthase and glucanosyl transferase mutants of A. fumigatus showed an altered GPI-anchored proteins pattern present in the mutant cell membrane compared to the wild type strain. This result suggests that a number of GPI-anchored proteins identified in this study may be involved in *A. fumigatus* cell wall biosynthesis and / or remodeling.

128 **Genomes to phenomes: genome scale mutagenesis and function analysis in the filamentous fungi.** Jeffery R. Shuster, Kiichi Adachi, Matthew Tanzer, Sanjoy Mahanty, Lakshman Ramamurthy, Maria V. Montenegro-Chamorro, Clive Lo, Rex Tarpey, Amy Skalchunes, Ryan Heiniger, Sheryl Frank, Blaise Darveaux, Todd Dezwaan, Grant Nelson, and Lisbeth Hamer. Paradigm Genetics Inc., 108 Alexander Drive, RTP, NC 27709.

The filamentous fungi include a number of organisms that have significant effects in the areas of human health and nutrition. Some fungi are major pathogens of the world's food crops while other are causal agents of human diseases. Fungi are also employed as biological factories producing a number of important metabolites such as such as antibiotics and other pharmaceutical compounds. They are powerful producers of many industrial enzymes and organic acids. Although there is a paucity of full genome information for the filamentous fungi, recent advances in DNA-based technologies allow for full functional genomic analyses. A fungal phenome may be defined as the total discernable phenotypes arising from a genome wide mutagenesis program. A method to define the fungal phenome will be described. The method is comprised of a high throughput mutagenesis method, Transposon Arrayed Gene Knock Out (TAG-KO^{TM}), a bioinformatics pipeline, and a broad spectrum high throughput phenotypic analysis program. **1**

129 **Analysis of ESTs from two time of day-specific libraries of *Neurospora crassa* reveals novel clock-controlled genes.** Minou Nowrousian[1], Hildur V. Colot[1], Hua Zhu[2], Doris Kupfer[2], Gloria Berrocal-Tito[1], Hongshing Lai[2], Deborah Bell-Pedersen[3], Bruce A. Roe[2], Jennifer J. Loros[1], Jay C. Dunlap[1]. [1]Dartmouth Medical School, Genetics and Biochemistry, Hanover, NH, 03755. [2]University of Oklahoma, Chemistry & Biochemistry, Norman OK USA. [3]Texas A&M University, Biological Sciences, College Station TX USA

In an effort to determine genes which are expressed in mycelial cultures of *Neurospora crassa* over the course of the circadian day, we have participated in a collaborative effort to sequence 13,000 cDNA clones from two time of day-specific libraries (morning and evening library) generating approximately 20,000 sequences. Contig analysis allowed the identification of 445 unique sequences and 986 genes present in multiple cDNA clones. For about 50% of the sequences, significant matches to sequences in the NCBI database (of known or unknown function) were detected. The remaining ESTs showed no similarity to previously identified genes. EST sequences and results of comparisons can be obtained from the following web site: http://www.genome.ou.edu/fungal.html. We hybridized Northern blots with probes derived from 26 clones chosen from contigs identified by multiple cDNA clones. Our results indicate that the representation of genes among the morning and evening sequences, respectively, in most cases does not reflect their expression patterns over the course of the day. Nevertheless, we were able to identify

four new clock controlled genes. Based upon these data we predict that a significant proportion of Neurospora genes may be regulated by the circadian clock. The 1431 different genes which are represented among the ESTs from both libraries represent an estimated 10% of all Neurospora genes. They may be used for further large scale analyses such as microarray approaches to elucidate the extent of clock control in *Neurospora crassa* .

130 **Identification of stage specific proteins in Phytophthora spp.** Pieter van West, Shuang Li, Samantha Shepherd, and Neil A.R. Gow. University of Aberdeen, Department of Molecular & Cell Biology, IMS, Foresterhill AB25 2ZD, Aberdeen, Scotland.

Pathogens of the oomycete genera *Phytophthora* cause destructive diseases of hundreds of commercially important plant species. Despite their economic importance, little is known about the molecular mechanisms accounting for the success of oomycetes as plant pathogens, or the fundamental molecular processes underlying their development. Oomycetes have many fungus-like characteristics, but are not true-fungi. A number of studies have indicated that they should be classified as Stramenopiles that include the golden-brown algae (Kamoun et al., 1999, TIPS 4: 195-200). This implies that oomycetes have distinct genetic and biochemical mechanisms involved in the interaction with plants that may be significantly different from those in true fungi. Oomycetes have several clearly defined developmental stages in their life cycle. They produce biflagellated zoospores that are able to swim in water surfaces on plant tissues and in the soil, enabling the pathogen to select its infection site. The zoospore encysts, forms a germ tube, from which an appressorium is produced that enables the pathogen to penetrate the plant surface. All these stages of the life cycle are experimentally tractable since they can be produced in vitro (van West et al., 1998, FGB 23, 126-138). A proteomics approach is being employed to identify stage-specific and extra-cellular proteins from two economically important *Phytophthora* species, *P. infestans* and *P. palmivora*. Our aim is to characterise stage specific and secreted proteins at the molecular level. Such proteins might play essential roles in the development of *Phytophthora* species.

131 **Analysis of transcriptional control circuits and their gene targets using transcript profiling in *Candida albicans***. Christophe d'Enfert[1], A. Munir A. Murad[2], Fredj Tekaia[3], Driss Talibi[4], Hélène Tournu[2], Daniel Maréchal[4], Claude Gaillardin[5], and Alistair J.P. Brown[2] . [1]Unit de Physiologie Cellulaire and [3]Unité de Génétique Moléculaire des Levures, Institut Pasteur, 75724 Paris Cedex 15, France; [2]Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, United Kingdom; [4]Eurogentec, Parc Scientifique du Sart Tilman, 4102 Seraing, Belgium; [5]Laboratoire de Génétique Moléculaire et Cellulaire, CNRS URA1925 INRA UMR216 INA-PG, 78850 Thiverval-Grignon, France

Saccharomyces cerevisiae Tup1 is a general transcriptional repressor which is targeted to specific promoters through an interactions with the specific DNA-binding proteins, including *Sc*Nrg1 and *Sc*Mig1. Inactivation of the homologue of *Sc*Tup1 in the human pathogen *Candida albicans* results in a constitutive filamentous growth which is an important determinant of *C. albicans* virulence (Braun and Johnson, Science 277:105, 1997). Inactivation of *Ca*Nrg1 also causes constitutive filamentous and invasive growth in *C. albicans* while a filamentous phenotype has not been associated to the inactivation of *Ca*Mig1 (Zaragoza *et al*. J. Bacteriol. 182:320, 2000). In this study, we have used transcript profiling to dissect the roles of *Ca*Tup1, *Ca*Nrg1 and *Ca*Mig1. Using the public data of the *C. albicans* genome sequencing program (www-sequence.stanford.edu/group/candida/index.html), 2002 open reading frames (ORFs) were identified and PCR products corresponding to their 3' end were spotted on nylon membranes. Hybridization profiles were obtained using RNAs prepared from *C. albicans* wild-type, *tup1/tup1*, *nrg1nrg1* and *mig1/mig1* strains and compared. Our results show that *Ca*Nrg1 and *Ca*Mig1 co-regulate separate but overlapping subsets of *Ca*Tup1-regulated genes, consistent with *Ca*Nrg1 and *Ca*Mig1 being *Ca*Tup1 targetting proteins. In particular, *Ca*Nrg1 regulates known hypha-specific genes and other virulence factors while *Ca*Mig1 regulates many metabolic functions. Genes with unknown functions identified as targets of *Ca*Nrg1 or *Ca*Mig1 are likely to participate in these same processes. In this regard, transcript profiling represents a powerful approach to identify genes that are potentially relevant to the virulence of *C. albicans*.

132 **Annotation and gene expression analysis of BAC clone 6J18 on chromosome 7 of *Magnaporthe grisea***. Woobong Choi[1], Youngjin Koh[2], and Ralph A. Dean[1]. [1]Fungal Genomics Lab. North Carolina State University, Raleigh, NC, USA. [2]Sunchon National University, Sunchon, Korea.

A 112 kb BAC clone located on chromosome 7 of the rice blast fungus, *Magnaporthe grisea*, was sequenced and annotated. Sequencing was accomplished in two phases. In the first or production phase, the BAC clone was fragmented into 1-2 kb fragments and cloned into pUC18. These clones were sequenced at random using M13 forward primer to produce ~8-fold coverage. In the second or finishing phase, the other ends of selected clones were sequenced using M13 reverse primer. Gene prediction annotation software, GenScan, identified 36 putative ORFs. 21 of these showed significant matches to known proteins as revealed by a BlastP search. Analysis of ESTs for *M. grisea* chromosome 7 identified 13 genes that mapped onto the BAC clone. Genomic Southern blot analysis revealed that all 13 genes existed in single/low copy number in the rice blast genome. Northern blot analysis showed that several genes are differentially expressed during appressorium formation. Sequence alignment of the BAC against the rice blast BAC-ends sequence (STC) database identified 70 neighboring BACs, the majority of which were found in the same fingerprint contig as assembled by the software program FPC. These results demonstrate that the sequencing BAC clones when combined with expressed sequence tags (ESTs) and BAC end sequence (STCs) analysis is a powerful and practical approach for elucidating the organization and function of the rice blast genome.

133 **Gene expression during growth and microsclerotia development in the vascular wilt pathogen *Verticillium dahliae*.** M.J. Neumann and K.F. Dobinson. Agriculture and Agri-Food Canada, 1391 Sandford St., London, ON Canada N5V 4T3, and Department of Plant Sciences, University of Western Ontario, London, ON Canada N6A 5B7

Vascular colonization by *Verticillium dahliae* is characterized by both filamentous and budding (yeast-like) growth. This dimorphic growth pattern, characteristic of vascular wilt fungi, is thought to be essential for the systemic colonization of xylem vessels. In the late stages of disease the pathogen enters a limited saprophytic growth phase, obtaining nutrients from the dying plant, and producing microsclerotia. cDNA libraries were constructed from cultures of *V. dahliae* grown either in a simulated xylem fluid medium (SXM), or under conditions that favour near-synchronous development of microsclerotia (MS). Up to 45% of the expressed sequence tags (ESTs) from approximately 1000 randomly chosen clones from each library had weak or no similarity to protein sequences in the NCBI databases. ESTs with significant scoring pairs (BlastX scores >50; E values $<10^{-7}$) have been assigned to functional groups. Homologues of melanin biosynthetic genes are present in the MS library, and not in the SXM library. A number of ESTs from the SXM library had high similarity to genes from other phytopathogenic fungi that are expressed preferentially *in planta*, and/or under nutrient conditions that favour pathogenic growth. These data suggest that the two libraries are representative of different phases within the life cycle of *V. dahliae*, and will be useful for further study of microsclerotial development and pathogenic growth. A detailed comparison of gene expression during the two growth phases will be presented, and the significance of the data discussed.

134 **Transposon impala from *Fusarium oxysporum*: a novel tool for tagging genes and genome analysis in ascomycetes.** M. J. Daboussi[1], S. Demais[1], A. Hua-Van[1], C. d'Enfert[1], A. Firon[1] , M. C. Grosjean-Cournoyer[2], T. Langin[1], M. H. Lebrun[2], M. G. Li Destri Nicosia[1], C. Masson[1], C. Scazzocchio[1], and F. Villalba[2]. [1]IGM and IBP, Universite Paris-Sud, Orsay, France. [2]+Biotechnologies and UMR CNRS-Aventis, Aventis CropScience, Lyon, France; Institut Pasteur, Paris, France.

impala is an active transposable element of *Fusarium oxysporum*. The imp160 copy, integrated into the niaD gene encoding nitrate reductase, behaves as an autonomous element since it excises from niaD and reinserts at a new genomic location in fungal isolates lacking active elements. A defective impala element with the transposase gene replaced by the hygromycin resistance gene was used to demonstrate the absence of endogenous transposase in impala-free isolates and the ability of different genomic copies of impala to promote transposition in trans. Autonomous and engineered impala copies were introduced in different fungal species such as *F. moniliforme*, an endophytic fungus, *Aspergillus nidulans*, a model ascomycetal fungus, *A. fumigatus*, a human pathogenic fungus, and *Magnaporthe grisea*, a plant pathogenic fungus. In all these species, impala transposes with a good efficiency in a manner similar to that observed in *F. oxysporum*. Analysis of a collection of strains with transposed impala elements shows that it can inactivate and tag genes including pathogenicity genes. The ability of impala to function in a two-component system and to transpose in different fungal species demonstrates its potential as a transposon tagging tool in fungi. Furthemore, analysis of the behavior of impala in different species or genetic backgrounds should give significant insights on the factors that modulate transposition efficiency.

135 **Genome wide analysis of *Cryptococcus neoformans*.** Steen, B.R., Tangen, K., MacDonald, K., Lian, T.S., *Marra, M., *Jones, S. Kronstad, J.. Biotechnology Lab, University of British Columbia. *Genome Sequence Centre, B.C. Cancer Agency.

Cryptococcus neoformans is an important opportunistic human pathogen. The development of new strategies to control cryptococcal infections will be greatly facilitated by a more detailed understanding of the genome of *C. neoformans*. We are taking a genome wide approach to discover and characterize *C. neoformans* genes involved in signaling and virulence in the context of adaptation to growth conditions in the host environment. For example, we are using serial analysis of gene expression (SAGE) to study the ability of *C. neoformans* strains H99 (serotype A) and JEC21 (serotype D) to adapt to growth at 37ºC. Four SAGE libraries were prepared representing each strain and each temperature. Because of the availability of the genome sequence (~3X shotgun coverage), we have initially focused our analysis on the two SAGE temperature libraries for strain JEC21. We have obtained 2,935 and 1,159 sequence reads for the JEC21 25ºC and 37ºC libraries, respectively and 1,561 and 1,948 sequence reads for the H99 25ºC and 37ºC libraries, respectively. From these, we have derived a total of 68,284 tags, 13,836 of which are unique, for the JEC21 25ºC library, 19,064 total tags, 7,310 of which are unique, for the JEC 21 37ºC library, 42,358 total tags, 10,179 of which are unique for the H99 25ºC library and 61,976 total tags, 11,298 of which are unique for the H99 37ºC library. These libraries are being compared to identify which genes are more highly expressed at infective temperatures (37ºC) compared to noninfective temperatures (25ºC). In addition, we are constructing physical maps of *Cryptococcus* in order to compare the serotype A strain with the serotype D strain. Digestion of 3000 BAC clones (*Hind*III digested) produced fingerprints which resulted in 27 contigs for H99 and 42 contigs for JEC21. Overgo hybridization for JEC21 and H99 specific sequences is being done in order to sew together the existing contigs and for comparison of the physical maps for the two strains. As well, these high resolution maps will aid in assembling the Stanford genome sequence for strain JEC21. These studies should contribute to a global understanding of this fungal pathogen and aid in identifying genes important in virulence.

136 **Adaptation of a PCR-based cloning method for use in filamentous fungi.** Michael Nielsen, Uffe H Mortensen, and Alexei Aleksenko. Technical University of Denmark, BioCentrum, Kgs. Lyngby, Denmark

The rapid accumulation of genomic sequences from a broad range of organisms fuels the need for specific genome manipulation. A cloning-free PCR based method for allele replacement exists where PCR products are fused together to produce two tailored DNA fragments suitable for co-transformation and subsequent integration into the genome of a given host. The fusion is accomplished by the use of adaptamers, which are PCR primers with overhangs that differentially tag the 5' and 3' of the amplified substrate. Complimentary adaptamers of two denatured PCR fragments anneal, thus fusing the products prior to PCR amplification of the whole fragment. Using this technique one fragment is generated that contains sequences matching the desired site of integration fused to the 5' 2/3 of a selectable gene and another where the integrative sequences are fused to the 3' 2/3 of the same selectable gene. After transformation, homologous recombination in the cell fuses the two fragments to reconstruct the entire selectable marker and inserts the fragments into the genome at the desired site. If a counter selectable marker is used, it can be excised from the genome by a direct repeat recombination event, leaving only the desired genomic alteration. So far this technique only been used in *Saccharomyces cerevisiae* because its genomic sequence is known, but as more genomic sequences become available the approach may become applicable to other organisms. To demonstrate this, we have adapted the method for use in the filamentous fungus, *Aspergillus nidulans*, by using it to replace the wild type yA allele with a mutant allele.

137 ***Agrobacterium tumefaciens*-mediated transformation of *Magnaporthe grisea*.** Hee-Sool Rho, Seogchan Kang[1], and Yong-Hwan Lee. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea. [1]Department of Plant Pathology, The Pensylvania State University, University Park, PA 16802, USA

Agrobacterium tumefaciens-mediated transformation (ATMT) has long been used to transfer genes to a wide variety of plants and has also served as an efficient tool for insertional mutagenesis. ATMT was established in the rice blast fungus, *Magnaporthe grisea*, to identify genes important for pathogenicity. Employing two binary vectors, carrying the bacterial hygromycin B phosphotransferase under the control of the *Aspergillus trpC* promoter as a selectable marker, led to the production of about 300 hygromycin B- resistant transformants per 1 x 10^7 conidia of *M. grisea*. Transformation efficiency correlated with the number of conidia used and the duration of co-cultivation with *Agrobacterium* cells. All transformants tested remained mitotically stable, maintaining their hygromycin B

resistance after several generations of growth in the absence of hygromycin B. Genomic Southern blot analysis showed that over 60% of the transformants contained a single T-DNA insert per genome. Considering the efficiency and flexibility of this ATMT protocol, ATMT appears to be a highly efficient alternative to other insertional mutagenesis techniques in characterizing those genes important for the pathogenicity of *M. grisea* and potentially that of other fungal pathogens.

138 **Identification of virulence and morphogenetic factors in *Histoplasma capsulatum* using a shotgun DNA microarray.** Lena Hwang and Jasper Rine. University of CA, Berkeley, Molecular Cell Biology, Berkeley, CA. USA

Histoplasma capsulatum is a dimorphic, pathogenic fungus that infects humans, causing pulmonary and systemic disease. It exists in mycelial form in the soil, and when aerosolized, the conidia are inhaled by the host. At 37C, within the host, *H. capsulatum* undergoes a phase transition from mycelia to yeast and is phagocytosed by macrophages. This organism survives within the phagolysosome and is capable of inhibiting the acidification of that compartment. In addition, *H. capsulatum* is also able to modulate the pH of its media to near neutral when grown in culture. In collaboration with Anita Sil at U.C. San Francisco, we have created a 9600-element *H. capsulatum* DNA microarray. The microarray contains random genomic fragments, representing approximately 1/3 of the genome. We have used the array to identify genes that are specifically expressed in either the yeast or mycelial form. The microarray is also being used to identify genes that respond to changes in the pH of the media. Genes that display a pH-dependent transcriptional response will be further characterized for their role in the modulation of pH in the phagolysosome of macrophages, as well as their role in virulence.

139 **Linkage disequilibrium is associated with an inversion in the *het-6* region of *Neurospora crassa*.** Cristina O. Micali, N. Mirrashed and M. L. Smith. Carleton University, Ottawa, ON. Canada

In *N. crassa*, heterokaryon incompatibility occurs upon fusion of individuals that differ any one of 11 *het* loci or the mating-type locus. Among these loci, allelic differences at *het-6* cause one of the most dramatic incompatibility reactions, resulting in death of heterokaryotic cells. The *het-6* region was previously shown to contain two distinct incompatibility genes, *un-24* (ribonucleotide reductase large subunit) and *het-6*. Two alleles, Oakridge (OR) and Panama (PA), have been identified at each locus. The two genes are in severe linkage disequilibrium; only OR/OR or PA/PA combinations were detected in a survey of more than 150 strains from nature. This suggests the *un-24-het-6* region acts as a *het*-gene complex. Here we compare the structure of the PA and OR forms of this region. PA strains carry a ~20 kbp inversion with respect to OR strains. PCR/RFLP markers provide evidence for recombination within and around the inverted region. DNA sequence was determined across ~12 kbp. Sequences at the inversion breakpoints are divergent in OR compared to PA strains. We hypothesize that these physical differences reduce recombination events that would give OR/PA or PA/OR combinations at *un-24* and *het-6*, respectively, and that the inversion polymorphism is maintained by association with the *het*-gene complex.

140 **Genome-scale gene identification, disruption and function assignment in filamentous fungi.** Lisbeth Hamer, Kiichi Adachi, Maria V. Montenegro-Chamorro, Matthew Tanzer, Sanjoy Mahanty, Clive Lo, Rex W. Tarpey, Amy Skalchunes, Ryan Heiniger, Sheryl Frank, Blaise Darveaux, Ted Slater, Lakshman Ramamurthy, Todd Dezwaan, Grant Nelson, Jeffery Shuster & Jeff Woessner. Paradigm Genetics Inc., 104 Alexander Dr., Bldg. 2, RTP, NC 27709 USA

The filamentous fungi are a large group of diverse and economically important microorganisms. Because of their complex genomes and a low rate of targeted integration during transformation, high throughput gene disruption and functional analyses are not readily applicable to these organisms. We have developed TAGKO™, a genome scale functional analysis approach to rapidly identify, disrupt and determine the function of genes without any prior sequence information. We show recent results using this technology in the pathogenic fungi *Magnaporthe grisea* and *Mycosphaerella graminicola*, the cause of rice blast and wheat blotch, respectively.

141 **Genomic efforts to study the plant pathogen *Fusarium graminearum*.** Linda Harris, Hélène Rocheleau, Sharon Allard, Tricia Glassco, Anju Koul, Thérèse Ouellet. Eastern Cereal & Oilseed Research Centre, Agriculture & Agri-Food Canada, Ottawa, Ontario, Canada K1A 0C6

Fusarium graminearum (teleomorph: *Gibberella zeae*) is a broad host pathogen, attacking a range of plant species including wheat and barley (head blight/scab), and maize (gibberella ear and stalk rot). It has been the cause of several recent epidemics causing millions of dollars in lost revenue to Canadian and U.S. cereal producers and has been identified as the most important cereal problem in Canada in terms of yield and grade losses and toxicity of infected grains. We are using a genomics approach to identify and characterize genes from *F. graminearum* which may be involved in the host-pathogen interaction and in fungal pathogenicity. Our goal is to build a large collection of ESTs which will be used to develop micro arrays. Several cDNA libraries have been constructed from *F. graminearum* at various developmental stages and under different growth conditions. EST sequencing from those libraries has been initiated. Our progress on this project will be presented.

142 **MycoPath™: a new resource integrating protein-specific information for the major fungal pathogens of humans**. Maria C. Costanzo, Laura S. Robertson, Glenn D. Krumholz, Janice E. Kranz, Pinar Kondu, Kevin J. Roberg-Perez, and James I. Garrels. Proteome, Inc., Beverly, MA, USA.

Information relevant to specific proteins of any particular fungal pathogen is found not only in the biological literature about that pathogen, but also in the literature concerning similar proteins of other fungi. Extracting and integrating information from all of these sources can be a formidable task for an individual researcher. To address this need, Proteome, Inc. maintains a growing database, the BioKnowledgeÖ Library, which contains extensive and up-to-date gene- and protein-specific information collected and organized by expert curators and editors. Four volumes of the Library (http://www.proteome.com/databases) are concerned with fungi and model organisms: YPD™, PombePD™, WormPD™, and CalPD™, covering the proteomes of *S. cerevisiae, S. pombe, C. elegans,* and *C. albicans,* respectively. The next volume to be released will be MycoPathPD, which will incorporate CalPD and add information about genes and proteins of several important human pathogens: *Aspergillus* species (*fumigatus, flavus,* and *niger;*) *B. dermatitidis;* several *Candida* species in addition to *albicans; C. immitis; C. neoformans; H. capsulatum;* and *P. carinii.* While complete genomic sequences are not yet available for any of these organisms, compiling all available sequence and literature information into one resource with the *C. albicans* sequences, in a format that facilitates comparisons with the *S. pombe* and *S. cerevisiae* proteomes, yields a valuable resource for antifungal research. Furthermore, the availability of this gene-indexed, searchable, and interlinked collection of fungal protein-specific information will greatly facilitate prediction of the functions of proteins encoded by newly sequenced fungal genomes.

143 **High throughput transformation and gene disruption of the phytopathogenic fungus *Mycosphaerella graminicola.*** Kiichi Adachi, Grant Nelson, Rex W. Tarpey, Maria V. Montenegro-Chamorro, Sheri Frank, Todd Dezwaan, Amy Skalchunes, Ryan Heiniger, Blaise Dareaux, Sanjoy Mahanty, Matthew Tanzer, Lisbeth Hamer, Lakshuman Ramamurthy and Jeffery Shuster. Paradigm Genetics, Inc., RTP, NC, USA

The Ascomycete fungus *Mycosphaerella graminicola* is a causal agent of Septoria leaf blotch of wheat. Despite intensive research, gene manipulation techniques such as DNA-mediated transformation and gene disruption are not well established for this fungus. Here, we present a highly efficient electro -transformation system and gene disruption data for *M. graminicola*. The electroporation conditions were optimized using a hygromycin B-resistant marker and transformation efficiency increased up to 100 transformants/1x10^7 protoplasts/ g DNA. Using this method we successfully disrupted *LEUC* (3-isopropylmalate dehydrogenase) and *ATR2* (ABC transporter) genes for pilot experiments. Targeted integration frequency was 4.3% for *LEUC* with 3.1 kb total flanking length, and 5.3% for *ATR2* with 6.7 kb total flanking length. To improve targeted integration frequency we invented TAGKO™ (transposon-arrayed gene knockout) technology to generate gene disruption vectors, which always contain long flanking regions of homologous DNA (~40 kb). High throughput gene disruption experiments of *M. graminicola* were initiated using TAGKO™ constructs. TAGKO™ technology increased targeted integration frequency up to 28%.

144 **Searching for coding regions in *Neurospora crassa* using a simple codon bias algorithm and consensus sequences**. Judith Galbraith[1,2], Dr. Don Natvig[1], Dr. Mary Anne Nelson[1], Dr. Laura Salter[1], Cara Slutter[1,2]. [1]University of New Mexico, [2.]Albuquerque High Performance Computing Center

Current Gene Finding Algorithms depend largely on elevated GC content in coding regions, and hexanucleotide or dicodon counts. Many of these tools are tailored for model organisms such as humans or yeast, and perform poorly with other species such as the filamentous fungus *Neurospora crassa*.

To locate coding regions in sequences that have no similarity to known genes in the public databases, characteristics distinctive of *Neurospora* have been examined. One such characteristic is the exaggerated difference between the counts of cytosine(C) and adenosine(A) residues in the third position of codons. This difference is measured using a log ratio, abbreviated log (C/A).

Since *Neurospora* has many short exons at the 5 prime ends of specific genes, other quantitative measures must also be employed for full gene prediction. The Kozak consensus sequence which surrounds the start codon and splice site consensus sequences flanking introns, with frequencies specific to *Neurospora*, are evaluated for each potential exon. The current algorithm does an exhaustive search for all possible exons using either a Kozak pattern or a 3 prime splice site as a beginning of coding and a 5 prime splice site or STOP codon as the end. Scoring has been done on all conserved patterns and on the content or codon bias of each putative exon. Logistic regression has been performed to test the significant effect of each descriptive variable on the binary response variable (coding or non-coding). A linear relationship is derived from this analysis and a probability value assigned for each putative exon. Then testing has been done for sensitivity and specificity. A web based tool has been designed and is available at: http://www.ahpcc.unm.edu/Research/CompBio/GeneFinder/NEX/

145 **Molecular evolution and comparative function characterization of the septin gene family.** Jiong Zhao and Michelle Momany. Department of Botany, University of Georgia, Athens, Georgia 30602

The septins are members of a highly conserved protein family that was first identified in budding yeast as proteins associated with the neck rings. Members of the septin gene family have been found in budding and fission yeast, filamentous fungi, fruit fly, worm, mouse, and man. Despite the differences in protein sequences, most of septins seem to be essential in both fungal and animal cells. Septins were initially thought to play a role in controlling cytokinesis, as evidenced by their concentration at the mother-bud neck in budding yeast and at the cleavage furrow in dividing animal cells. However, recent data suggest that they may function in a much wider array of contexts such as cell surface organization and vesicle fusion processes. In this study we identified and compared 55 septin genes from fungi and metazoa. Our phylogenetic analysis indicates that most septins can be distributed into five orthologous classes, which include two fungal-specific classes, one animal- specific class and two mixed fungal-animal classes. Based on intron-exon comparison, most fungal septins do not show paralogs in the same species. However, most of animal septins have paralogs. Based on our phylogenetic analysis, we suggest function for some uncharacterized septins.

146 **Variants among Neurospora: an analysis of alternative splicing in *Neurospora crassa.*** Patricia L. Dolan, Kathryn J. Gruchalla, Diego Martinez, Gary Montry, Donald O. Natvig,Mary Anne Nelson. University of New Mexico

Alternative splicing of precursor mRNAs is an important mechanism for regulating gene expression and generating protein diversity. It allows the selection of different splice sites to produce different transcripts and sometimes variant proteins from a single gene. Alternative pre-mRNA splicing is a widespread phenomenon, occurring in organisms as diverse as the nematode *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster*,and humans. Evidence of alternative splicing has been found for about one-third of human genes, with the majority of alternative forms occurring from splicing events in the 5ÆÆ untranslated regions. A study to estimate the extent of alternative splicing in *Neurospora crassa*pre-mRNA has been undertaken. Using TIGR Assembler and Phrap EST assemblies, and where possible subsequent matching of ESTs to genomic sequences, alternative splicing variants have been identified for a number of genes. The issue of alternative splicing versus inefficient splicing (or partially processed nuclear intermediates) is discussed. With continued accumulation of *Neurospora* EST and genomic data, further insights into the patterns of alternative splicing will be possible. Identifying those proteins with variant forms will allow a greater and more complete understanding of the expression of *Neurospora's* proteome.

147 **Simultaneous mapping of multiple *vic* loci in *Gibberella fujikuroi* MP-A.** K. A. Zeller[1], J. E. Jurgenson[2], and J. F. Leslie[1]. [1]Dept. of Plant Pathology, Kansas State University, Manhattan, KS; [2]Dept. of Biology, University of Northern Iowa, Cedar Falls, IA.

We have used AFLP markers to map *vic* (vegetative incompatibility) loci that segregate in the mapping population of *Gibberella fujikuroi* MP-A [*Fusarium verticillioides* (=*F. moniliforme*)]. We crossed two *nit1*- strains from the mapping population's parents genetic backgrounds, and selected for recombinant progeny with vegetative compatibility (VC) to a strain of known AFLP genotype. This selection for VC should simultaneously select for identity in all segregating *vic* loci, and will skew segregation ratios for markers linked to each locus. We have used AFLP fingerprints from 104 progeny to define QTL-like associations between markers and VC phenotype. We have examined segregation of > 240 mapped polymorphic AFLP markers generated with 22 selective primer combinations. From these data we have identified 9 unlinked genomic regions with segregation ratios that deviate significantly (alpha < 0.05) from 1:1. In at least two of these regions we have identified AFLP markers that map within 2-3 map units of putative *vic* loci. These data will allow us to rapidly isolate and characterize *vic* loci from *G. fujikuroi* MP-A, and will give us a model for understanding VC interactions among other, less genetically tractable, members of *Gibberella* and *Fusarium*.

148 **Microarray analysis of *frequency* - regulated gene expression in *Neurospora crassa*.** Zachary A. Lewis[1], Alejandro Correa[1], Xie Xin[2], Daniel J. Ebbole[2] and Deborah Bell-Pedersen[1]. [1]Dept of Biology, Texas A& M University. [2]Dept of Plant Pathology, Texas A&M Univeristy

The filamentous fungus *Neurospora crassa* is a well-established model system for the study of circadian rhythms. The clock gene *frequency (frq)* was identified through classical genetic studies and has since been shown to be an important component of the pacemaker machinery. *frq*mRNA and FRQ protein levels oscillate over the course of the day, and FRQ is believed to either directly or indirectly control rhythmic processes in the fungus. Consistent with this possibility, known clock-controlled genes have been shown to be transcriptionally depressed or elevated in frq-null strains. We are using a transcriptional profiling approach to identify candidate genes that are positively or negatively regulated by FRQ protein. A total of 1800 clones from the University of New Mexico EST library have been arrayed on glass slides. These arrays have been probed with cDNA made from RNA isolated from *frq*+ and *frq* ^{10} *(frq-null)* cultures harvested at the time of peak FRQ levels. Currently, we are optimizing hybridization conditions. In addition,we are performing Northern analysis to confirm the results obtained from array hybridization. This approach should identify components of the FRQ regulatory pathway along with other pacemaker components. Candidate genes will be further characterized by Northern analysis, gene inactivation, and analysis of mutant phenotypes.

149 **Systematic identification of essential genes in the human pathogenic fungus *Aspergillus fumigatus*.** A. Firon[1], M.-C. Grosjean-Cournoyer[2], A. Beauvais[3] and C.d'Enfert[1] . [1]Unit de Physiologie Cellulaire, Institut Pasteur, Paris, France,[2] Biotechnology Department, Aventis Crop Science, Lyon, France and [3]Unit des Aspergillus, Institut Pasteur, France.

Invasive aspergillosis has become the most frequent air-borne fungal infection in patients with a deficient immune system. Because of a difficult diagnosis and the lack of efficient antifungal treatments, it is associated with a mortality rate as high as 85%. Invasive aspergillosis is mostly due to *Aspergillus fumigatus*, an opportunist pathogen. Despite intensive efforts, no genuine virulence factors have been identified in this species suggesting that virulence is a multifactorial process. Another way to identify novel antifungal targets is to define *A. fumigatus* genes that are essential for fungal growth . Here, we have developed a strategy combining insertional mutagenesis and parasexual genetics for the identification of essential genes in *A. fumigatus*. Although *A. fumigatus* is haploid and devoid of a sexual cycle, we were able to generate heterokarions using haploid *pyrG* strains with complementary auxotrophic and spore-colour markers and to identify stable diploid strains. Disruption of one allele of the *AfFKS* gene encoding 1,3-beta-D-glucane synthase in this strain resulted in a heterozygous diploid which did not produce haploid progenies upon benomyl-induced haploidization when the selective pressure corresponding to the *AfFKS* disruption was maintained. This result demonstrated that beta 1,3 glucan synthesis is an essential process for growth in *A. fumigatus*. Furthermore, it suggested that the identification of heterozygous diploids unable to produce haploid progenies could be used to define essential genes in *A. fumigatus*. A collection of heterozygous diploids was obtained by insertional mutagenesis of the diploid strain with a *A. nidulans pyrG*-bearing plasmid using

electroporation of intact conidia. 8% of these heterozygous diploids were unable to produce haploid progenies when the selection for the *pyrG* gene was maintained during haploidization. When haploid progenies were obtained in the absence of a selective pressure, none had a pyrG+ phenotype, suggesting that in all cases tested insertion had occured in an gene essential for *A. fumigatus* growth. Molecular analysis, using a semi-random PCR strategy, of heterozygous diploids containing a disrupted essential gene will be presented. Our data show the potential of using heterozygous diploids to demonstrate the essentiality cloned *A. fumigatus* genes or to identify on a random basis genes that are essential for *A. fumigatus* growth.

150 **Phospholipid induced extracellular protein analysis in *A. fumigatus*.** Michael J Bromley, Mike Birch, Jayne L Brookman. School of Biological Sciences, University of Manchester, England.

Aspergillus fumigatus is the most common mould infection of humans world-wide with disease incidence increasing particularly amongst immuno-compromised individuals. Current treatments for *A. fumigatus* infection are limited and resistant strains have been identified.

Functional analysis of *A. fumigatus* currently relies on genetic techniques that have been adapted from those successfully employed in prokaryotes and yeasts. Unfortunately it has become clear that these technologies do not provide the high throughput analysis necessary for an economically viable search for new drug targets. Proteome analysis using two-dimensional gel electrophoesis may provide the answer.

Comparative proteome analysis has been performed on *A. fumigatus* samples grown in the presence/absence of a phospholipid which is the primary constituent of lung tissue at the initial site of infection in invasive pulmonary aspergillosis. We have identified several proteins that are unique to the secreted complement of *A. fumigatus* proteins when grown in the presence of the phospholipid. Detailed analyses of these proteins will be presented.

151 **Analyses of ESTs and promoters of useful expression from *Aspergillus oryzae.*** Hiroko Hagiwara, Motoaki Sano, Sumiko Kunihiro, Kumiko Takase, Midori Yamamoto, Masayuki Machida. Natl. Inst. Biosci. & Human-Technol., Tsukuba, Ibaraki, Japan

Aspergillus oryzae is an important filamentous fungus in Japanese beverage and fermentation industries, and for the production of industrially valuable enzymes. To accumulate the basic knowledge of nucleotide sequences and expression of individual genes, we randomly sequenced cDNA libraries, yielding 5' expressed sequence tags (ESTs). The entire project was done by collaboration of Natl. Res. Inst. of Brewing, Natl. Food Res. Inst., Tohoku Univ., Tokyo Univ. Agric. Technol., Nagoya Univ. and Univ. Tokyo, using libraries from several different culture conditions including solid state culture, alkaline condition and at germination. The total number of the analyzed ESTs and contigs after clustering were approximately 17000 and 6000, respectively. We estimated that partial sequences of roughly a half of the total *A. oryzae* genes, which are estimated to be 8000-9000, had been sequenced. The total lengths of the contigs reached 4.7 Mb, which was equivalent to approximately 16% of the *A. oryzae* genome. In the present work, we focused on ESTs from liquid culture with and without carbon source consisting of 2478 and 1790 ESTs, respectively. These ESTs are expected to be useful as references to ESTs from other culture conditions. Database search by the BLAST algorithm showed that approximately 40% of the contigs had no similarity with any genes or proteins registered in the public databases to date. We have started sequencing promoters of strong expression and induction, which are important for protein production, and promoters of industrially valuable genes. Functional analyses of the promoters in bioinformatics and biological approaches will facilitate the understanding the genetic network in *A. oryzae*, which should enable a flexible control of this organism in the industrial application. A part of the above data is available from our web site (http:///www.aist.go.jp/RIODB/ffdb/index.html).

152 **Chromosomal polymorphisms, gene mapping and ploidy of *Paracoccidioides brasiliensis*.** Luciano dos Santos Feitosa[1], Màrcia R. Machado dos Santos[1], Renato A. Mortara[1], Jos Franco da Silveira[1], Patrícia S. Cisalpino[2], Zoilo Pires de Camargo[1].[1]Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, São Paulo, Brasil. [2]Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

Paracoccidioides brasiliensis (anamorph; teleomorphic stage unknown), a thermo dimorphic fungus, is the ethiological agent of paracoccidiodomycosis (PCM), a human granulomatous disease prevalent in Latin America. Genetic composition and genomic organization of *P. brasiliensis* is poorly understood. We worked on the electrophoretic karyotype (PFGE, as in Cano et al., 1998) of twelve fungal isolates from different geographic areas obtained from patients with chronic and acute PCM, armadillo and soil. Stable and reproducible karyotypes were observed. Our results were consistent with a haploid number of 4 - 5 chromosomal bands in the range of 2,5 to 10,0 Mpb. The haploid genome was estimated to be 24-30 Mbp. Six distinct karyotypic profiles were observed. Chromosomal polymorphisms made it difficult to correlate the banding pattern among isolates. In order to determine chromosome identity, nine specific gene probes have been used to hybridize Southern blots containing intact chromosomal bands. One genetic linkage group and eight distinct profiles were observed. There was a significant regularity when matching genes to chromosomes within certain size range, suggesting that the overall structure of the genome could be quite constant. We also estimated the DNA content of DAPI-stained nuclei of the twelve isolates by confocal microscopy. Estimation of the genome sizes by PFGE and microfluorimetry indicated the possible existence of both haploid and diploid fungal isolates. Supported by: PRONEX, FAPEMIG, FAPESP.

153 **Transcript profiling during ectomycorrhiza development.** Tomas Johansson, Antoine Le Quéré, Dag Ahrén, Joakim Lundeberg, Rikard Erlandsson, Bengt Söderström, Anders Tunlid. Lund University, Microbial Ecology Lund, Sweden. and Royal Institute of Techn., Biochemistry & Biotechn., Stockholm Sweden

Mycorrhiza, a symbiotic interaction between plant and fungi, is found on a vast majority of all plant species including many important forest trees and crop plants. The symbiotic interaction, which involves the development of specific infection structures in the plant rhizosphere, improve the growth of the host plant by facilitating nutrient uptake from the soil, while the fungus gains carbohydrates from the plant. The developmental process is anticipated to depend on a well-tuned regulation at both temporal and spatial levels, and the objective of this research is to isolate and characterize symbiosis-related (SR) genes by analyses of gene expression during the ectomycorrhizal development between the fungus Paxillus involutus and the plant Betula pendula (birch). Several cDNA libraries have been constructed from symbiotic ectomycorrhizal tissue, from free-living hyphae of P. involutus and from non-infected roots of B. pendula, respectively. So far we have obtained 5 -end EST sequence information from approximately 10,000 EST clones. The annotation and assignment of putative cellular function(s) is still in progress, but score frequencies from database comparisons yields significant differences between libraries. It is obvious that the transcript profile of the mycorrhiza is significantly different from that of the fungus and the plant, and clones are here observed to be of both fungal and plant origin. Among the high-score transcripts there are a number of fungal and plant genes (ôôcandidatesöö) that has been suggested to be involved in the development and function of the mycorrhizal symbiosis or in the interaction between fungal pathogens and plants.

154 **Smart man genomics: Expression profiling of organisms with unsequenced genomes.** Ted van der Lende, Cees van den Hondel and Frank Schuren. Department of Applied Microbiology and Gene Technology, TNO Nutrition and Food Research, P.O. Box 360, 3700 AJ Zeist, The Netherlands.

In our view the basis of genomics is the holistic, non-targeted study of in principle all biomolecules in an organism. This can be at the level of RNA (transcriptomics), proteins (proteomics) or metabolites (metabolomics). In our lab we have fully implemented the infra-structure for expression profiling (transcriptomics) of organisms of which the entire nucleotide sequence is available. In addition, we developed the ôôsmart man genomicsöö concept, a strategy which circumvents full genome sequencing but nevertheless enables transcriptome analysis of in principle every microorganism. We show that, in spite of the dogma that one needs a full genome sequence of an organism to study transcription profiles, expression profiles of organisms with unsequenced genomes is feasible. An explanation of this strategy and some preliminary data will be presented.

155 **Microbial Metabolomics**. Mariët J. van der Werf* and Cees A.M.J.J. van den Hondel. Department of Applied Microbiology and Gene Technology, TNO Nutrition and Food Research, P.O. Box 360, 3700 AJ Zeist, The Netherlands.

Applied genomics technologies (transcriptomics, proteomics, and metabolomics), aimed at analyzing the keystone biomolecules of a cell in its entirety, have ushered a new era in scientific methodology in the life sciences. Metabolomics is the most recent addition to the applied genomics toolbox. In analogy with transcriptomics and

proteomics, metabolomics involves the non-targeted, holistic determination of changes in the complete set of metabolites (low molecular weight molecules) in the cell (the metabolome) in response to environmental changes. Subsequently, knowledge is gained by analyzing different metabolomes using bioinformatic tools (pattern recognition) that either identify metabolites relevant for a specific environmental condition or interpret the quality/status of the complete metabolome (cluster analysis). Moreover, the information obtained from a metabolomics experiment gives information as to which metabolic pathways are used by the organism or even that are functioning in a specific compartment. This is of special importance when working with (metabolically) poorly characterized organisms. Metabolomics is expected to become of crucial importance for metabolic engineering, as it can be applied to determine bottle necks in biosynthesis. Due to the intrinsic characteristics of the metabolomics technology (measured metabolite concentrations, and its holistic and comparative approach), it is expected to replace metabolic flux analysis as the primary tool for deciding were to change a metabolic network in order to improve productivity. Moreover, metabolomics can be applied for growth medium optimization for increasing microbial growth rates or productivity, to determine the key molecules that induce (desired or undesired) secondary metabolite or enzyme production, to predict the effect of individual medium components on the overall flavour profile, to establish regulatory networks, and to predict the quality of the end-product form the quality of the microbial starter culture in food fermentations.

156 **Sequencing of the centromeric region of chromosome V in *Podospora anserina* : a draft project for the complete sequence of the genome**. Christian Barreau[1], Alain Billault[2], Laurence Cattolico[3], Robert Debuchy[4], Simone Duprat[3], Sebastien Kicka[4], Annie Sainsard-Chanet[5], Carole H. Sellem[5], Philippe Silar[4] and Béatrice Turcq[1]. [1]Institut de Biochimie et Génétique Cellulaires, UPR CNRS 9026, 1 rue Camille Saint-Saens, F-33077 Bordeaux, France. [2]Centre d'Etude du Polymorphisme Humain, 75010 Paris, France. Present address: Molecular Engines Laboratories, 20 rue Bouvier, F-75011 Paris, France.[3]Génoscope, Centre Nationale de Séquenéage, 2 rue Gaston Cr mieux, CP 5706, 91057 Evry Cedex, France. [4]Institut de Génétique et Microbiologie, UMR 8621, bâtiment 400, Université Paris-Sud, F-91405 Orsay cedex, France. [5]Centre de Génétique Moléculaire, UPR CNRS 9061, bâtiment 24, Allee de la terrasse, F-91190 Gif sur Yvette, France .

Podospora anserina is used as a model system for investigations about translation, control of sexual development, stability of mitochondrial genome, mitochondrial metabolism, non-conventionnal infectious elements or vegetative incompatibility. Each of these fundamental processes is controled by a large array of genes. The analysis of these processes will beneficiate from the access to the complete genomic sequence of *P. anserina*. In addition, comparative genomic using *P. anserina* genome sequence should yield interesting results about the evolution of fungal species. A BAC library has been constructed in the vector pBHYG, which is derived from pBeloBAC11 by the addition of a selectable hygromycine resistance gene in *P. anserina*. Several BAC which contain sequences from the locus of the centromere of chromosome V have been isolated and their sequencing is in progress. Preliminary results suggest that the region which is genetically defined as the centromere of chromosome V is not present in the library. Several genes present on either sides of the centromere have been identified in the BAC sequences. The progress of the work can be monitored on http://www.cns.fr/ or http://cgdc3.igmors.u-psud.fr/.

157 **AFLP linkage map of *Gibberella zeae***. J. E. Jurgenson[1], R. L. Bowden[2], K. A. Zeller[2], J.F. Leslie[2], N. A. Alexander[3], and R. D. Plattner[3]. [1]Department of Biology, University of Northern Iowa, [2]Department of Plant Pathology, Kansas State University, [3]Mycotoxin Research Unit, USDA/ARS National Center for Agricultural Utilization

A genetic linkage map of *Gibberella zeae* (*Fusarium graminearum*) was constructed by crossing nitrate nonutilizing (nit) mutants of *G. zeae* strains R- 5470 (from Japan) and Z-3639 (from Kansas). Ninety- nine nit+ progeny were selected and analyzed for polymorphisms using AFLP markers. Thirty-one pairs of two-base selective primers revealed 1044 polymorphic markers that mapped to 444 unique loci on nine linkage groups. The total map length of the genome from this analysis was 1247 centimorgans with an average interval of 2.8 map units between loci. Three linkage groups had high levels of segregation distortion. Selection of nit+ recombinant progeny accounts for two of the skewed regions. One linkage group had an abundance of symmetrical crossovers and appeared to have an intercalary inversion. Loci governing red pigment, trichothecene toxin amount, toxin type (deoxynivalenol vs. nivalenol), and the Tri5 gene were mapped.

158 **High throughput mutation by targeted disruption in the rice blast fungus, *Magnaporthe grisea*.** Grant Nelson, Rex W. Tarpey, Kiichi Adachi, Maria V. Montenegro-Chamorro, Sheri Frank, Todd Dezwaan, Amy Skalchunes, Ryan Heiniger, Blaise Dareaux, Sanjoy Mahanty, Matthew Tanzer, Lisbeth Hamer, Lakshuman Ramamurthy and Jeffery Shuster. Paradigm Genetics, Microbial Department, RTP, NC, USA

Paradigm Genetics is industrializing the process of gene function determination. The Microbial Research Group has developed a functional genomics platform to explore fungal genomes and uncover gene function across an array of economically important organisms. The fungus *M. grisea* is the causative agent of rice blast disease and is responsible for severe economic losses worldwide. Like most of the filamentous fungi, the frequency of homologous recombination in *M. grisea* is generally low. Our functional genomics platform utilizes TAGKO™ (transposon-arrayed gene knockout) technology to rapidly generate 'knock out vectors' of the entire genome. Furthermore, all of these cosmids contain flanking regions of ~40 kilobases. Subsequent sequencing and bioinformatics provide large numbers of targets with corresponding knock out vectors. To determine the function of these targets, we have developed a method for high throughput *in vivo* disruption. This method is based upon maximizing the frequency of homologous recombination in filamentous fungi and rapidly screening transformants to identify knock out mutants. Data will be presented, illustrating that transformation with TAGKO™ vectors dramatically increases the frequency of targeted disruptions in *M. grisea*.

159 **Functional genomics of *Phytophthora sojae*: identification of a necrosis inducing peptide by a heterologous expression assay.** Dinah Qutob[1, 2], Mark Gijzen[1] and Sophien Kamoun[3]. [1]Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, ON, Canada; [2]Department of Microbiology and Immunology, University of Western Ontario London, ON, Canada; [3]Department of Plant Pathology, The Ohio State University-OARDC, Wooster, OH, USA, 44691.

Phytophthora sojae is an oomycete that causes stem and root rot on soybean plants. To discover pathogen factors that activate plant defense responses, we identified putative secretory proteins from a database of expressed sequence tags (ESTs) (Plant Physiol. 2000 May;123(1):243-54) and assayed selected candidates using a potato virus X (PVX)-based heterologous expression system. First, we screened translational reading frames within ESTs for signal peptide motifs. From an analysis of 3035 ESTs originating from mycelium, zoospore, and infected soybean tissues, we identified over 300 putative proteins with N-terminal transit peptides. We further analyzed the candidates for a series of features of the encoded proteins, including size, hydrophobicity, putative glycosylation, similarity to known proteins, and completeness of the open reading frame. We selected a total of 17 different cDNAs encoding putative secreted proteins ranging in size from 5-24 kD for expression analysis in Nicotiana benthamiana using PVX/Agrobacterium-based assays. The results indicated that recombinant PVX expressing two of the 17 candidates caused necrosis and browning symptoms atypical of wild-type PVX infection. One cDNA was especially active, causing both localized and spreading tissue damage of infected plants. This necrosis-inducing peptide shares sequence similarity to other proteins found in fungal and bacterial species and may be involved in virulence of *P. sojae*.

160 **Identification of a hypervirulent mutant and genes of mitochondrial origin incorporated into the nuclear genome of *Cryptococcus neoformans*.** Nelson, R. T., Handly, L. A., Hua, J., and Lodge, J. K.. Saint Louis University, Doisy Department of Biochemistry and Molecular Biology, St. Louis, MO. 63104

Cryptococcus neoformans is a yeast-like fungus with a world wide distribution. *C. neoformans* causes cryptococcosis which is a threat to a large patient population with impaired immune function. We have identified a signature tagged insertional mutant which is able to produce approximately 10 fold more CFUs in the brain of mice in a disseminated model of cryptococcosis. The virulence of this mutant does not appear to be a result of increased resistance to the reactive oxygen intermediates nitrous oxide or hydrogen peroxide relative to the parental strain. Analysis of the flanking genomic sequence indicates that the insertion interrupts the 5' UTR for a mitochondrial ND4L gene on one side and sequence that has no match in the existing databases on the other side. CHEF analysis of the insertion site, indicates that this site is in the nuclear genome on a chromosome of approximately 1.2 Mb. Southern blot of CHEF gels of the parental strain, H99 used in the signature tagged mutagenesis, indicates that a number of mitochondrial genes are also present in this chromosome. This is the first report of hypervirulence and the first report of mitochondrial genes present in the nuclear genome of *Cryptococcus neoformans* var. *neoformans* strain H99.

161 **Targeted gene inactivation by homologous recombination in *Cryptococcus neoformans* var. *neoformans* serotype A using linear DNA and biolistic delivery.** Nelson, R. T. and Lodge, J. K.. Saint Louis University, Doisy Department of Biochemistry and Molecular Biology, St. Louis, MO 63104

Cryptococcus neoformans var. *neoformans* is a opportunistic pathogen of immunocompromised individuals. Homologous recombination in *Cryptococcus* has been studied mainly in serotype D strains. In order to systematically investigate the parameters for homologous recombination and targeted gene inactivation in *C. neoformans* serotype A, we constructed a number of DNA fragments with varying lengths of sequence (400, 300, 200, 100 and 50 bp) from a serotype A *CAP59* gene flanking a selectable marker. Disruption of this gene inhibits capsule polysaccharide synthesis producing an acapsular phenotype. These molecules were transferred into the genome of *Cryptococcus* using biolistic transformation followed by analysis of phenotype and genotype by PCR and Southern blot. The results indicate that as little as 200 bp of homologous sequence on each flank could specifically target the inactivation of the *CAP59* gene with an efficiency of 19%. Increasing the length of the flanking sequence to 400 bp increased the efficiency to 54%. Asymmetrical constructs were created with 400 bp of flanking sequence on one side of a selectable marker and 50 bp on the other. These constructs were as effective (6-22)% in inactivating the *CAP59* gene as the symmetrical molecules containing 200 bp of homologous sequence. These results indicate that linear molecules with as few as 200 bp of homologous sequence on both sides of the selectable marker can be used to effectively knock out genes in *C. neoformans* serotype A and that a short flanking sequence on one side of a selectable marker can be complemented by a longer flanking sequence on the other flank.

162 **Chromosome landing across avirulence loci in the potato late blight pathogen, *Phytophthora infestans.*** Stephen Whisson[1], Theo van der Lee[2], Glenn J Bryan[3], Robbie Waugh[3], Francine Govers[2], and Paul RJ Birch[1] . [1]Scottish Crop Research Institute, Unit of Mycology, Dundee, Scotland. [2]University of Wageningen, Phytopathology, Wageningen The Netherlands. [3]Scottish Crop Res. Inst., Unit of Genomics, Dundee Scotland

Phytophthora infestans, which causes late blight of potato and tomato, is a globally important plant pathogen. On potato, resistance to late blight is conditioned by a gene-for-gene interaction between potato resistance (R) genes and *P. infestans* avirulence (*Avr*) genes. Six *P. infestans Avr* genes have been genetically mapped and AFLP markers have been identified tightly linked to these *Avr* loci. We have adopted a map-based cloning approach for cloning the *Avr* genes from *P. infestans*. A 10-fold genome coverage bacterial artificial chromosome (BAC) library was constructed and clones were pooled for screening by AFLP. Overlapping BAC clones which span two avirulence loci (*Avr11, Avr4*) have been identified, and BAC clones near two other *Avr* genes (*Avr1, Avr2*) have also been identified. Current and future efforts will be focussed on confirming the presence of *Avr* genes in the BAC clones, and identifying the functional *Avr* genes. Cloning of an *Avr* gene from *P. infestans* will yield insights into the molecular interaction between *P. infestans* and potato, and may form a basis for novel sources of resistance to late blight.

163 **A comparative study of *Aspergillus fumigatus* genes.** Anderson MJ, Prebble EJ, Denning DW. University of Manchester, Dept of Medicine, Manchester, Gr Manchester, UK

A comparative analysis of all the published *Aspergillus fumigatus* genes has been carried out as an aid for the *A. fumigatus* genome sequencing project. This project was initiated in July 1999 and as of December 2000, funding has been obtained to sequence 50 % of the genome. Funding for the remainder is currently being sought. Two sets of analyses have been performed on the full-length *A. fumigatus* genes published in the literature or in the DNA databases (~60). Consensus sequences have been determined for the initiation codon and for the 5' and 3' splice sites of introns. These consensus sequences were generated using only those sequences which had been confirmed experimentally. Intron splice site sequences were compiled only in those examples where both the sequence of the gene and mRNA had been determined and for the initiation codon, sequences were complied where the position had been determined by N-terminal protein sequencing, transcriptional start site mapping or by comparison of gene and mRNA sequences. These consensus sequences should be useful for programming gene-finding software. The second set of analyses compared *A. fumigatus* gene sequences with similar sequences in other Aspergilli to determine the level of conservation in the structure of homologous genes. Similar sequences were identified by searching the fungal directory of the EMBL database using Fasta. These sequences were aligned and the size, position and number of exons were compared. Some interesting examples will be presented including the comparison of catalases,

polyketide synthases, chitin synthases and proteases. This type of analysis might assist in determining which homologous genes are most closely related to each other and therefore help define orthologues.

164 ***Agrobacterium tumefaciens*-mediated targeted gene disruption in *Coccidioides immitis.*** Kris Osborn[1], Marc J. Orbach[2], Anath Das[3], Maria Lourdes Lewis[4] and John N Galgiani[1]. [1]University of Arizona, Infectious Diseases, Tucson, AZ. [2]University of Arizona, Plant Pathology, Tucson AZ USA. [3]University of Minnesota, Biochem,Mol Biol,Biophys, St. Paul MN USA. [4]Southern AZ VA Healthcare, Research, Tucson AZ USA

Coccidioides immitis, the causative agent of Valley Fever, is a dimorphic fungal pathogen of humans and other mammals. To develop targeted gene disruption in *C. immitis* we used *A. tumefaciens*-mediated transformation to delete the gene for Ag2/PRA, a spherule wall protein of unknown function which stimulates protective immunity against coccidioidal infection. To create a knockout cassette, a 1.4 kb modified hygromycin B phosphotransferase gene (HygR) under the control of the *Aspergillus nidulans* trpC promoter was used to replace the Ag2/PRA gene. This construct was placed between the left and right borders of an *A. tumefaciens* binary vector to create pKO322. *A. tumefaciens* ($5X10^8$ cells) harboring pKO322 were co-cultivated with $5x10^6$ germinated (24 hour) arthroconidia for 3 days, after which the germlings were plated on 2XGYE containing 20 g/ml hygromycin and 50 g/ml kanamycin. At least 50 discrete HygR *C. immitis* colonies grew in each of 3 experiments. Selected colonies were screened by PCR. The results of this screen, together with the observation that more than 90% of arthroconidia contain two nuclei, raised the concern that transformed strains were heterokaryons. We passaged arthrocondia from 26 putative transformed strains under more stringent selective conditions to isolate homokaryons. Homokaryons from eleven separate transformation events were recovered. In nine of these, the Ag2/PRA gene appeared to be knocked out as evidenced by a PCR product predicted for replacement of the locus by the HygR cassette and no detectable Ag2/PRA signal. Thus, the rate of homologous integration for this gene was high enough to be readily observed in a single transformation experiment.

165 **High-throughput gene disruption in *Ashbya gossypii* reveals genes required for normal filamentous fungal growth.** Thomas Gaffney, Krista Gates, Keri Cavanaugh, John Marhoul, Natasha Springer, Melisa Harrison, and Michelle Kirksey. Syngenta-RTP, Research Triangle Park, NC, USA

The filamentous ascomycete *Ashbya gossypii* is a useful model organism in a functional genomics approach due to its small genome size (ca. 9 megabases encompassing ca. 5000 genes) and the relative ease with which gene replacement experiments can be conducted. Also, yeast replicons function in *Ashbya*, allowing complementation of mutant constructs. Since the *Ashbya* genome has not undergone the extensive duplication observed in the *Saccharomyces* genome, it is often more straightforward to link phenotype with genotype in the *Ashbya* background. Our studies with *Ashbya* have revealed numerous genes whose effect on filamentous growth would have been difficult to predict based upon analysis of related *Saccharomyces* genes. Also, we have found that several *Ashbya* homologues of *Saccharomyces* genes required for yeast pseudohyphal growth do not influence *Ashbya* filamentous growth.

166 **Identifying pathogenicity and essential genes in fungi by deletion scanning.** Jianguo Wu, Barbara Robbertse, Xun Wang, Olen Yoder and Gillian Turgeon. Novartis Agricultural Discovery Institute, San Diego, California, U.S.A.

Knowledge of the fungal genes essential for life and those controlling molecular mechanisms of pathogenicity would suggest both fungicide targets and strategies by which plants resistant to disease might be developed. We are taking a genome-wide approach to the identification of such genes in *Cochliobolus heterostrophus*, a pathogen of maize. The project involves three elements: (1) Sequencing the fungal genome; (2) Directed mutagenesis aimed at evaluating candidate genes whose products are suspected of being involved in fungal pathogenesis, and (3) Saturation mutagenesis of the genome. For the third goal, a library designed to delete small random fragments from the genome was constructed and transformed into a wild type strain. Each transformant is tested for viability and pathogenicity. Mutants with either altered virulence or lethality are noted and the plasmid used for transformation sequenced, permitting the deleted DNA to be identified in each case. To validate the result and to identify the gene(s) responsible for the phenotype of interest, each ORF affected by the deletion is targeted individually. A pilot study indicated that mutations affecting viability and virulence could be detected by this strategy.

167 **Genome characterization of three plant pathogenic ascomycetes.** Paolo Amedeo[1], Scott Baker[1], Todd Moughamer[2], Don Hutchison[3], John Thompson[2], Trini Miguel[3], Hemant Varma[2], Darrell Ricke[2], Chris Martin[2], Stephen Goff[3], Olen Yoder[1], and Gillian Turgeon[1] . Novartis Agricultural Discovery Institute Inc., 3115 Merryfield Row, San Diego, CA, 92121-1125 [1] Plant Health [2] Bioinformatics [3] Structural Genomics

The genomes (~35 Mb) of three phylogenetically diverse plant pathogens (*Cochliobolus heterostrophus*, *Gibberella zeae/Fusarium graminearum* and *Botrytis cinerea*) have been sequenced to different extents using a shotgun approach. Each has been characterized individually and by comparison. The data allow us to make comparisons to genomes of other organisms such as the single celled, fungal non-pathogens *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, the multicellular, filamentous, non-pathogens *Neurospora crassa* and *Aspergillus nidulans*, and to other filamentous pathogens. Inter-organism comparisons should allow us to sort genes essential for life, for filamentous growth, and for pathogenicity. The latter is expected to be subdivided into two categories: those common to all or most pathogens, and those unique to particular pathogens with specific host ranges.

168 **Genetic analyses of Iprodione resistant mutants of *Cochliobolus heterostrophus* and cloning of histidine kinase genes.** Akira Yoshimi, Chihiro Tanaka and Mitsuya Tsuda. Pesticide Research Institute, Faculty of Agriculture, Kyoto University, Kyoto 606-8502, Japan.

We have isolated Iprodione resistant mutants of *Cochliobolus heterostrophus*, and identified mutant genes at three loci; Ipr1, Ipr2 and Ipr3 by crossing experiments. Strains carrying mutant alleles at Ipr1 or Ipr2 showed Iprodione resistance (MIC>400 g/ml) and sensitive to high osmolarity (ED50 for KCl, 300 400mM), whereas wild type's MIC for Iprodione was 2.5 g/ml and ED50 for KCl was ca. 600mM. At Ipr3, we have distinguish two types mutant allele by their ED50 for KCl. One type showed ED50 similar range of ED50 to the Ipr1 or Ipr2, the other allele showed highly sensitivity to high osmolarity (ED50, 25 200mM). These were indicating that the osmotic stress responding genes in the pathways such as histidine kinase and MAP kinase would be involved in the Iprodione resistance in this fungus. We made an attempt to clone histidine kinase by a PCR approach. Based on the consensus amino acid sequences in the histidine autokinase domain, the nucleotide-binding domain and ATP-binding domain, degenerated primers were designed and used to amplify genomic fragments of *C. heterostrophus*. Three kinds of fragments from putative histidine kinase genes (bmhk-1, bmhk-2 and bmhk-3) were obtained. Nucleotide sequence of bmhk-1 and bmhk-2 showed high similarities to Nik-1 (os-1) from *Neurospora crassa*, Chk-1 from *Glomerella cingulata* , respectively.The bmhk-3 shared homology with LuxQ from *Vibrio cholerae*. Gene- disruption studies are currently underway.

169 ***Agrobacterium tumefaciens* T-DNA as a potential insertional mutagen for *Blastomyces dermatitidis* and *Histoplasma capsulatum.*** Thomas D. Sullivan, Peggy J. Rooney, Colin P. Kealey and Bruce S. Klein. University of Wisconsin, Pediatrics, Madison, WI, USA

B. dermatitidis and *H. capsulatum* are pathogenic dimorphic fungi responsible for pulmonary infections and disseminated disease. Random mutagenesis by insertion of known DNA sequences may aid in identification and cloning of virulence factors and other genes of these fungi. Integration of T- DNA from *A. tumefaciens* has been used in this way in plants, and recent studies indicate that *A. tumefaciens* can deliver DNA into fungal cells. Vectors containing hygromycin phosphotransferase or *URA5* selectable markers within T-DNA sequences were introduced into *A. tumefaciens. ura5* auxotrophs of *H. capsulatum* and newly isolated *ura5* auxotrophs of *B. dermatitidis* were used as targets for *URA5* marker selection. Cocultivation of *A. tumefaciens* and yeast-phase cells was followed by selection for hygromycin-resistant or uracil-prototrophic yeast. For auxotrophic strains of both fungi, transformation frequencies were greater than ten-fold higher using *URA5* selection than for hygromycin selection. Southern blot analyses confirmed that the T-DNA is often integrated at a single site in the genome, but at different sites in independent transformants. As *B. dermatitidis* yeast are multinucleate, we sought to obtain cells harboring the same mutation in all nuclei, facilitating isolation of recessive mutations during phenotypic screens. To this end, we also transformed germinating single- nucleate *B. dermatitidis* conidia by this method.

170 **Sequencing the *Cryptococcus neoformans* genome.** Eula Fung, Richard W Hyman, Don Rowley, Dan Bruno, and Ronald W Davis. Stanford Genome Technology Center, Stanford, CA USA

Cryptococcus neoformans is the etiologic agent of cryptococcosis, one of the most serious fungal diseases worldwide. *C. neoformans*' haploid genome is contained on 13 chromosomes and totals 21 Mb. Strain JEC21, serotype D was chosen for sequencing because it represents the only known isogenic mating pair. Our approach is a whole genome shotgun plus assembly. We will identify and finish as many open reading frames as possible, subject to the usual time and money constraints.

As a service to the research community, all of the raw shotgun sequence data and contigs-in-progress will be posted on an overnight basis on the Stanford Genome Technology Center website. An initial assembly of the data was done in late October 2000 when we had reached 3-fold coverage of the genome. The assembly includes the circular mitochondrial DNA and 3900 contigs covering almost 13 Mbases of the nuclear DNA. As of Dec 15, 2000, we have over 138,000 shotgun reads. Taken together, these reads provide almost 81 Mbases. *C. neoformans* (strain JEC21, serotype D) genomic DNA was generously provided by Dr. June Kwon-Chung, NIAID, NIH, USA. Our C. neoformans Genome Project is supported by a cooperative agreement (U01 AI47087) from the NIAID, NIH, USA. Funding commenced in March, 2000, and will run for three years.

http://www-sequence.stanford.edu

171 ***Aspergillus nidulans*** **genome sequencing effort: Strategic scaffold sequencing of Chromosome IV.** Patricia Ayoubi[1], Nigel Dunn-Coleman[2], Nancy Keller[3] and Rolf Prade[1]. [1]Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA. [2]Genencor International, Palo Alto, 925 Page Mill Road, CA, USA. [3]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA.

As a simple multicellular eukaryote, the ascomycete *Aspergillus nidulans* is an important model for addressing fundamental questions in cellular and molecular biology. *A. nidulans* has also become a model system for basic study of biotechnology and pathogenesis of related multicellular ascomycetous fungi. In an effort to generate publicly available genome sequence data of *A. nidulans*, we have started pilot scale sequencing of chromosome IV (2.9 Mb). Since the *A. nidulans* genome is estimated to contain ~8,000 genes over ~30 Mb, this sequencing effort is expected to reveal ~800 genes. Chromosome IV is covered by 83 non-overlapping ordered cosmids called tiles (average 40 kb inserts) with an additional 62 cosmids filling the gap between tiles. This minimum set of overlapping cosmids is a scaffold that provides the necessary framework for maximizing gene discovery from sequencing efforts. Our sequencing strategy involves subcloning (2 kb) and shotgun end-sequencing of about 180 subclones per cosmid. To anchor all cosmids known to hybridize to chromosome IV, 240 cosmids have also been end-sequenced. To date, approximately 25,000 successful reactions have been completed. Up to date progress on the assembly and sequence analysis of these data will be presented.

172 ***Aspergillus nidulans*** **EST microarrays.** Hugh Russell[1], Sven Krappmann[1], Patricia Ayoubi[1] , Nigel Dunn-Coleman[2], and Rolf Prade[1] [1]Department of Microbiology and Molecular genetics, Oklahoma State University, Stillwater, OK 74078 USA and [2]Genencor International, Palo Alto, 925 Page Mill Road CA 94304-1013 USA

The northern blot has been the standard for transcript monitoring for many years; however, with finished and current genome sequencing projects mounting, new large-scale gene expression analysis tools are needed. Microarrays are DNA/DNA hybridization devices in which ALL or a large number of gene-specific targets (DNA fragments) derived from a genome, have been cross-linked to a support (e.g., glass) and hybridized against labeled probes of high degree of complexity - e.g., reverse transcribed and labeled mRNA populations. DNA microarray gene expression monitoring has been useful in various applications including drug discovery, transcriptional regulated gene expression studies, and gene discovery. Here, we describe the construction of a glass-based microarray containing approximately 4,600 cross-linked targets derived from the *A. nidulans* expressed sequence tag (EST) collection. PCR amplified targets were copied from a cDNA library, primed by an EST-specific and a vector-common oligonucleotide set. Template targeted regions for amplification were chosen, whenever possible, to map to the 3''-end of the EST (ORF) and produce a 500 bp fragment. Multiple bands amplified with a single EST primer could in theory be utilized, if in the majority of cases they correspond to alternate 3' end terminations. However, we have determined that under our laboratory conditions, alternate 3'-end termination could not be established in PCR reactions showing two or more bands. Thus, PCR reaction which did not result in a single band, were redone using a secondary and/or a tertiary PCR cycling protocol. Finally, new primers for non- validated PCR products were

designed. All PCR-products were ethanol- precipitated, targets validated by agarose gel electrophoresis and printed onto glass-slides. Microarrays will be made available upon request by Genencor International and Oklahoma State University.

173 **Constructing and sequencing a BAC contig of the *Phytophthora sojae* genome.** Felipe Arredondo and Brett Tyler. Department of Plant Pathology University of California, Davis Davis, CA95616.

Phytophthora sojae causes $1-2 billion in damage to soybean crops worldwide each year. To facilitate isolation of pathogenicity genes from *P. sojae* by map-based cloning, we are constructing a BAC contig of the entire genome of this organism, using a hybridization fingerprinting strategy. We are hybridizing a 13X BAC library with unique mixtures of random probes, most of them repetitive, and with ESTs. Computer software has been developed to collect, simulate and analyze the data. At present we have probed the library with 49 of the mixtures. Each mixture hybridized to around 300 - 500 BACs. 19% of the BACs so far have received the minimum number of hits needed to establish statistically significant overlaps (5 each). Of these BACs, 34% have been placed into contigs. We have confirmed the authenticity of three of the largest contigs by HindIII digestion.

With the long term goal of sequencing the entire genome of *P. sojae*, we have begun preliminary sequencing of a 200 kb BAC contig spanning two avirulence genes from *P. sojae*. Sequencing of the first 60 kb BAC is nearly complete. In the region sequenced so far the gene density is extremely high. Both the sequencing data and the BAC hybridization data suggest that the *P. sojae* genome is composed of gene-rich regions separated by regions rich in repetitive sequences.

174 **EST analysis and sequencing of chromosome 7 of the rice blast fungus, *Magnaporthe grisea.*** T. Mitchell[1], T. Houfek[1], T. Mitchell[1], S. Martin[1], D. Ebbole[2], R. A. Dean[1]. [1]Fungal Genomics Laboratory, North Carolina State University, Raleigh, NC [2]Texas A&M University, College Station, TX

Magnaporthe grisea, the causal agent of rice blast disease, is not only the largest threat to rice production worldwide, but is recognized as a leading model system for fungal biology, genetics, and pathology. As part of an international effort, a major initiative has been funded to use a genomics approach for gene discovery and whole genome analysis of this pathogen. We are currently sequencing 35,000 5' ESTs from 8 cDNA libraries prepared from different stages of growth and development. A minimum set of 5,000 unique genes will then be sequenced from the 3' end to validate sequence data and contig assemblies. Concurrently, we are using a BAC-by-BAC approach to sequence chromosome 7 of this fungus. We will obtain ~5X coverage of the minimum tile of 41 BACs covering this chromosome. All EST and BAC sequence data will be publicly available on a BLAST server. Results from initial EST and BAC sequencing efforts will be presented as well as an analysis of sequence data for each part of the project.

175 **Durable resources for discovery and development of new gene products from *Trichoderma reesei*. Phase I cDNA and BAC end sequencing.** M. Chellappan[1], N. Dunn Coleman[2], A. Hillian[1], T. Houfek[1], T. Mitchell[1], P. van Solingen[3], P. Teunnissen[3], D. Wang[2], M. Ward[2], J. Yao[2], R. A. Dean[1]. [1]Fungal Genomics Laboratory, North Carolina State University, Raleigh, NC [2]Genencor International, Palo Alto, CA [3]Genencor International, Leiden, The Netherlands

The filamentous fungus *Trichoderma reesei is* a major producer of cellulytic and other enzymes used in commercial applications. The discovery of new genes is critical to the continued development of commercially competitive biologically based products. In this study, two cDNA libraries were constructed, one with RNA extracted from cells grown in conditions promoting the production of cellulytic enzymes, and the other from cells grown in 18 different conditions. A total of 9,792 ESTs were sequenced using ABI 3700 automated sequencing system. Of these, 6,603 were identified to be of high quality (excluding those clones with no inserts) using criteria to be presented. Using Phrap 4,267 clones were placed into 1,207 contigs leaving 2,336 singlets. Each of the two libraries had the same level of redundancy while the redundancy for the entire project stands at 46%. The total number of unique sequences identified is 3,543. Sequence data was subjected to BLASTX and N similarity searches and processed through an automated gene-indexing package. Approximately half of sequences had significant matches. A *HindIII* BAC library has been constructed and clones are being sequenced from the ends. Results from cDNA and BAC end sequencing,

BLAST searched, and indexing will be presented. BAC filters, BAC clones, and cDNA clones will be made available to academic users via online requests.

176 **EST analysis for the discovery of new gene products from *Aspergillus niger*.** N. Dunn-Coleman[2], M. Chellappan[1], M. Conboy[2], A. Hillian[1], T. Houfek[1], T. Mitchell[1], P. van Solingen[3], P. Teunnissen[3], D. Wang[2], M. Ward[2], J. Yao[2], R. A. Dean[1]. [1]Fungal Genomics Laboratory, North Carolina State University, Raleigh, NC [2]Genencor International, Palo Alto, CA [3]Genencor International, Leiden, The Netherlands

Many fungi, particularly Aspergillus species, produce various secondary metabolites desirable for commercial development. An EST sequencing project was established to facilitate the discovery of new genes with potential for commercial applications. Two cDNA libraries were constructed using total RNA derived from different growth conditions such as nutrient limitation, stress response and alternative N, C sources. One library was normalized using a novel technique to be presented. Sequence results show an overall success rate of 80% with an average high quality read length of 462 bp. Sequence data has been subjected to BLASTX and N similarity searches and processed through an automated gene-indexing package. BLAST results will be presented and will be available in a searchable and browsable format on a publicly accessible web site. cDNA sequence and clones will be made available to academic users upon request.

177 **PipeOnline 2.00: data mining of processed DNA sequence databases.** Patricia Ayoubi[1], Eduardo Misawa[2] and Rolf Prade[1] . [1]Department of Microbiology & Molecular Genetics and [2]School of Mechanical & Aerospace Engineering, Oklahoma State University, Stillwater, OK 74078 USA.

The exponential increase in uncharacterized public genomic DNA and cDNA derived sequence data has driven the development of computational methods for processing data for the purpose of gene identification and inference of biological function. PipeOnline 2.00 is an experimental Web-based resource designed by the OSU Bioinformatics Laboratory to aid investigators in determining metabolic and other protein functions from large-scale DNA sequence collections. Demonstration databases were generated from public cDNA sequence data derived from fungal and plant model organisms. Typically, raw DNA sequence data (collection of trace or FASTA files) are processed automatically by PipeOnline 2.00, a series of script-linked programs which process raw DNA sequence files and produces a database of records that can be retrieved through queries, or comprehensive gene-function browsing. PipeOnline 2.00 produces contig-assembled files, assembled using publicly available software. These contigs are than automatically compared against a local NCBI non-redundant protein database using BLASTX and resulting output files automatically collected, parsed, formatted, assembled, indexed and uploaded to a local MySQL server. PipeOnline also automatically produces a functionally sorted output, facilitating analysis of large DNA sequence collections, specifically data sets with high levels of functional redundancy. Functional sorting was achieved through a proprietary sorting method that utilizes functional information gathered from public databases. The current version of PipeOnline 2.00 employs definitions and the biologically threaded functional tree derived from the Metabolic Pathways Database, MPW dictionary.

178 **Comparative studies of gene organization between *Magnaporthe grisea* and other fungal species.** Jun Seop Jeong, and Ralph A. Dean, Fungal Genomics Laboratory, North Carolina State University, Raleigh, NC 27695 USA.

Magnaporthe grisea causes rice blast disease, which results in devastating losses in rice production worldwide. Due to its significance, the genetics and physiology of the fungus have been studied extensively--It has become a leading model system for the investigation of host-pathogen interactions. Numerous resources including genetic maps, large insert BAC libraries, and ESTs provide a foundation for genomic studies. Chromosome 7 has been studied in most detail including the mapping of several hundred ESTs. Emerging sequence data from several fungal species, now, allow for comparative analysis of gene organization to be undertaken. Comparative studies have revealed syntenic relationships among higher eukaryotes, such as grasses and vertebrates. However, detailed comparison of genome organization among fungal species is lacking. cDNA clones from chromosome 7 of *M. grisea* were identified, and used to investigate syntenic relationships in *Aspergillus nidulans* and *Neurospora crassa* by sequence alignment and DNA hybridization analysis. This comparative approach will facilitate genome reconstruction efforts of filamentous fungi and provide insight into genome evolution and function.

179 **Genomic sequence comparisons and the role of small molecules in signaling in filamentous ascomycetes.** Scott Kroken, John. W. Taylor, and N. Louise Glass. Dept. of Plant and Microbial Biology, UC-Berkeley

Filamentous fungi make and secrete small molecules that may be involved in signaling, defense or scavenging nutrients. In collaboration with Olen Yoder and Gillian Turgeon at Syngenta, we are performing comparative genomics of saprobes (e.g. *Neurospora crassa*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) and plant pathogens (*Cochliobolus heterostrophus*, *Botrytis cinerea*, *Fusarium graminearum* and *Ashbya gossypii*). A comparative analysis is aimed at identifying genes that encode secreted ribosomal peptides, polyketides (PKs), and nonribosomal peptides (NRPs). We have cloned a 2530 amino acid-encoding gene from *N. crassa* that is similar to *PKS1* (38% identity), a polyketide synthase (PKS)-encoding gene involved in making a plant toxin (T-toxin) in *C. heterostrophus*. A *N. crassa* 5217 amino acid-encoding gene was identified that is similar to *HTS1* (33% identity), a nonribosomal peptide synthetase (NRPS)-encoding gene also involved in the production of a plant toxin (HC toxin, a cyclic tetrapeptide) in *C. carbonum*. Based on analysis of the as yet incomplete *N. crassa* genome sequence, it is predicted that *N. crassa* has 3-7 PKS and PS genes, in contrast to *C. heterostrophus*, which is predicted to have >35 PKS-encoding genes and >35 NRPS-encoding genes. Based on these comparisons, a mutational analysis of genes involved in the production of small molecules will be performed in *N. crassa*. Metabolite profiling between wild-type *N. crassa* and mutants will be performed to match PKS and NRPS genes with secreted metabolites. Our goal is to delineate the role of small molecules in signaling pathways that are required for formation of the hyphal network, sporulation, vegetative growth and response to environmental cues in filamentous fungi.

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180 ***Agrobacterium tumefaciens*-mediated expression of cry genes in *Trichoderma hazianum*.** Hoang Quoc-Khanh, Cao Cuong and Le Tan Duc. Institute of Tropical Biology, Microbiology, Ho Chi Minh City, VN, Vietnam

Agrobacterium tumefaciens transfers a part of its Ti plasmid, the T-DNA to plant cells during tumorigenesis. Not restricted to plant species, *A. tumefaciens* is able to transfer T-DNA to yeasts and filamentous fungi. A highly efficient and reproducible procedure for the transformation of the antagonistic *Trichoderma harzianum* by *A.tumefaciens*-mediated homologous recombination is reported. Transformation were carried out with plasmids carrying cry genes, which encode *Bacillus thuringiensis* insecticidal crystal proteins.

181**Heterologous protein production in *Aspergillus niger*.** Jesper Mogensen, Bettina Andreasen, and Hans van den Brink. Chr. Hansen A/S, Genetics, Hørsholm, Denmark

Chr. Hansen uses *Aspergillus niger* to produce bovine chymosin. To get more insight in the processes involved in chymosin secretion we decided to use GFP as a tool. Two constructs were made, both using the glucoamylase (glaA) promoter. In the first construct the gfp gene was directly fused to the glaA promoter, resulting in cytoplasmic localisation of GFP. In the second construct a glaA-gfp fusion gene, resembling the expression cassette used for chymosin production, was made. Both constructs were transformed to a chymosin producing *A. niger* strain and compared to a laboratory strain (AB4.1) carrying similar constructs. Studying these strains we made two interesting observations, which will be discussed on our poster; 1. In a single hyphal tip both active and non active

compartments can be found 2. GFP is liberated from the glucoamylase carrier intracellularly even though no processing site is present in the construct.

182 **Efficient production of *Arthromyces ramosus* peroxidase by *Aspergillus awamori*.** B. Christien Lokman, Vivi Joosten, Robin J. Gouka, Ernst-Jan Rutjes, Jacqueline Hovenkamp, and Cees A.M.J.J. van den Hondel. TNO Voeding, P.O. Box 360, 3700 AJ Zeist, the Netherlands.

Peroxidases are produced by a wide variety of organisms including plants, animals, and microorganisms. The specificity and biological functions of peroxidase vary with the source of the enzyme. *Arthromyces ramosus* peroxidase (ARP) is a 41 kD monomeric glycoprotein containing one mole of protoheme IX per mole of enzyme as a prosthetic group. ARP has a broad specificity for phenolic and anilinic hydrogen donors which makes the enzyme interesting for industrial applications. Until now, the heterologous expression of peroxidases has been studied only with limited success. In these studies heme availability has been suggested to be a possible bottleneck for the overproduction. In our study we analysed the expression of the gene encoding ARP in the filamentous fungus *Aspergillus awamori*. Under control of the endoxylanase promoter secretion of active ARP was achieved up to 0.6 g/L in shake flask cultures. Westernblot analysis showed an rARP product of the correct size. Furthermore, our results suggest that there is no heme limitation during overproduction of ARP in *A. awamori*.

183 **Cloning and expression of the cyanide-insensitive alternative oxidase gene (*aox1*) from a citric acid-producing strain *Aspergillus niger* WU-2223L.** Masashi Yoda, Kohtaro Kirimura, Kiyotake Kamigaki, Kuniki Kino, and Shoji Usami. Department of Applied Chemistry, School of Science and Engineering, Waseda University, Ohkubo 3-4-1, Shinjuku-ku, Tokyo, 169-8555, Japan.

In *Aspergillus niger* WU-2223L, a cyanide (CN)- and antimycin A- insensitive and salicylhydroxamic acid (SHAM)-sensitive respiratory pathway exists and is catalyzed by the alternative oxidase (AOX), besides the CN-sensitive cytochrome pathway. Both the CN-insensitive and SHAM-sensitive respiration and the activity of AOX, as duroquinol oxidase activity, were shown to be localized in mitochondria. Such respiration and specific activity in purified mitochondria increased by addition of methanol, an inducer of citric acid production, or antimycin A, both accompanying the increase of citric acid productivity. On the other hand, when WU-2223L was cultivated with SHAM, the AOX activity decreased accompanying the decrease of citric acid production drastically although mycelial growth was not affected. The complementary DNA (cDNA) and chromosomal DNA encoding the AOX were cloned. One full-length cDNA was obtained and sequenced to reveal that the clone contained an ORF encoding a polypeptide of 351 amino acid. The deduced amino acid sequences revealed that there are two long hydrophobic regions regarded as membrane-spanning regions of the protein and two iron-binding motifs as a reactive center. When the whole ORF was introduced and expressed in *Escherichia coli*, the transformant harboring pKAOX1 containing the ORF gene showed cyanide-insensitive and SHAM- sensitive respiration, and the expression was induced to two folds by addition of IPTG. The chromosomal DNA encoding AOX gene (*aox1*) was also cloned from a chromosomal DNA library of *A. niger* and a 2856 bp-long DNA fragment was isolated. The *aox1* contains two introns, and two TATA boxes in untranslated regions. Southern hybridization analysis with the cDNA and *aox1* as probes revealed that there is only one copy of *aox1* in the chromosome of *A. niger* WU-2223L. The motifs for carbon catabolite repressor (CREA) and two nitrogen metabolite repressor (AREA) binding sites were also found in upstream region of the ORF, suggesting that the carbon catabolism and nitrogen metabolism regulation might be involved in the transcription of *aox1*. The Northern blot analysis was done on the total RNA extracted from the mycelia cultivated under citric acid producing-conditions with 2% methanol (v/v). The transcription activity was highest for the mycelia cultivated for 2 days, the lowest for 4 days, and maintained at almost constant level through 6 to 8 days, and the correlation between the mRNA levels and AOX activities was observed.

184 **MstA: an *Aspergillus niger* high affinity transporter of glucose and mannose**. Patricia A. vanKuyk[1], Jasper Diderich[3], Andrew P. MacCabe[2], Oscar Hererro[2], George J. G. Ruiter[1], and Jaap Visser[1]. [1] Molecular Genetics of Industrial Microorganisms. Wageningen University. Dreijenlaan Nl-6703 HA Wageningen. The Netherlands. [2] Consejo Superior de Investigaciones Cientificas. Instituto de Agroquimica y Tecnologica de Alimentos. P.O. Box 73. Burjassot. Valencia 46100. Spain. [3] Swammerdam Institute for Life Sciences. BioCentrum. University of Amsterdam. Plantage Muidergracht 12. NL-1018 TV Amsterdam. The Netherlands.

A gene encoding a putative sugar transporter was cloned from an *A. niger* gDNA library using the *A. nidulans* putative sugar transporter, *mstA*, as a probe. The gene identified has been designated *mstA*$_{(A.\ niger)}$. The derived amino acid sequence of MstA has the highest similarity to the amino acid sequence of *Amantia muscaria* MST-1, as well as to a number of yeast sugar sensors including *Saccaromyces cerevisiae* Snf3. *mstA* is expressed under conditions of carbon starvation indicating that this gene is subject to carbon catabolite repression. Expression levels of *mstA* in a wildtype strain are influenced by extracellular pH. A full length cDNA clone of *A. niger mstA* has been isolated and sequenced. This cDNA has been inserted into a shuttle vector in which the expression of *mstA* is under the control of a copper inducible promoter that is functional in *S. cerevisiae*. After transformation into a *S. cerevisiae* strain in which a number of sugar transporters have been disrupted (hxt1delta-hxt7delta gal2delta) a copper inducible partial restoration of growth was observed when glucose, fructose, or mannose is the sole carbon source. These results imply that MstA$_{(A.\ niger)}$ is able to transport glucose, fructose, and mannose. Uptake experiments using ^{14}C labelled sugars as the substrate for the yeast strain expressing *mstA*$_{(A.\ niger)}$ confirmed that MstA transports glucose, fructose, and mannose. The Km value obtained for fructose was 4 mM, whilst the Km values for glucose and mannose are at least 10 fold lower. This result, in conjunction with the expression data, suggests that *A. niger* MstA is a hexose transporter with high affinity for glucose and mannose.

185 **A Fruitbody tissue method for efficient *Agrobacterium*-mediated genetic transformation of *Agaricus bisporus.*** Xi Chen, Michelle Stone, Carl Schlagnhaufer and C. Peter Romaine. Department of Plant Pathology, The Pennsylvania State University, University Park, Pennsylvania 16802 USA

We have devised a highly efficient and convenient *Agrobacterium*-mediated genetic transformation method for the mushroom, *Agaricus bisporus*. Transformation was carried out using a binary plasmid vector (pBGgHg) comprised of a pCAMBIA1300 backbone with the hygromycin resistance (*hph*) gene controlled by the *A. bisporus* glyceraldehyde 3-phosphate dehydrogenase promoter. Gill tissue pieces from fruitbodies were cocultivated for 3 days in the presence of acetosyringone with *A. tumefaciens* carrying pBGgHg, and then transferred to a hygromycin-containing medium. Antibiotic-resistant cultures appeared after 7 to 28 days from ca. 35% of the tissue pieces. The choice of promoter, bacterial strain and fruitbody tissue was critical for maximal transformation efficiency. Transformants had from one to four copies of the *hph* gene integrated at random sites in the genome. The antibiotic resistance trait was stably maintained, being expressed by the first-generation fruitbodies and basidiospores. Our method creates new prospects for using transgenic technology in the genetic improvement of this commercial mushroom, and represents an important tool for the genetic analysis of biological processes in this species.

186 **Expression of Mn-peroxidase gene from *Coriolus versicolor* in transgenic tabacco for remediation.** Iimura, Y.[1], Ikeda, S.[2], Sonoki, T.[2], Hayakawa, T.[3], Kinbara, K.[3], Kajita, S.[2], Katayama, Y.[2] and Tatsumi, K.[1]. [1] Natl Inst Res & Env, AIST, MITI, Hydrospheric Environ Prot, Tsukuba, Ibaraki 3058569, Japan; [2]Tokyo Univ Agri &Tech, BASE, Koganei Tokyo 184-8588 Japan; [3]Railway Tech Res Inst, , Koganei Tokyo 1858540 Japan

Plants offer many advantages over bacteria as agents for remediation; however, they typically lack the degradative capabilities of specially selected bacterial strains. Biodegradative abilities of plants are less impressive than those of adapted bacteria and fungi. But these disadvantages are balanced by the large amounts of plant biomass that can easily be sustained in the field. Transgenic plants expressing microbial degradative enzymes could combine the advantages of both system. We genarated transgenic tobacco expressing Mn-peroxidase gene from *Coriolus versicolor* .

187 **Isolation and transformation of uracil auxotrophs of the edible basidiomycete *Pleurotus ostreatus.*** Beom-Gi Kim, Young-Bok Yoo and Suk-Tae Kwon[1]. Division of Applied Microbiology, National Institute of Agricultural Science and Technology, Suwon, 441-707 Korea. [1]Department of Genetic Engineering, Sungkyunkwan University, Suwon, Korea.

Uracil auxotrophs of *Pleurotus ostreatus* were isolated using resistance to 5' fluoro-orotic acid (5'-FOA). Uracil auxotrophs obtained were transformed to prototrophy using plasmid pTRura 3-2 that contains the orotidine monophosphate decarboxylase (ura3) gene from *Trichoderma reesei*. Southern blot analyses of the transformants showed that the transforming DNA had integrated into the genome of the protoplasts. Normal fruiting bodies were induced in hybrid to cross uracil auxotroph with wild-type monokaryon, and the basidiospores collected from this

fruiting body showed a biased segregation rate to prototrophy. Uracil auxotrophic monokaryons were crossed each other and dikaryotic auxotrophic strains were generated. We will use these strains as host for transformation.

188 ***Hop*, an active *MuDR*-like element in the filamentous fungus *Fusarium oxysporum*.** Fabienne Chalvet[1], Fiona Kaper[1], Thierry Langin[2] and Marie-Josée Daboussi[1]. [1]Université Paris Sud, IGM, Orsay, France; [2]Université Paris Sud, IBP, Orsay France

Four different class II transposons have been identified in the genome of the fungal plant pathogen *Fusarium oxysporum*. All are active since they have been trapped through their transposition into the nitrate reductase structural gene (*niaD*) used as a target. Three belong to the *Tc1-mariner* superfamily, and the last one, presented here, is related to the *MuDR* family. Two insertions located in the last exon of the *niaD* gene, at the same nucleotidic site and in opposite orientation, have been characterized. These elements, called *Hop*, are 3299 bp long with perfect inverted terminal repeats of 99 pb. Partial sequencing of genomic copies reveals a 9 bp target site duplication and no apparent sequence specificity at the insertion sites. Sequencing of a cDNA indicates that *Hop* does not contain any intron and encodes a putative transposase of 836 AA. Comparison of *Hop* transposase to those from *MuDR*-like elements (maize, rice, *Arabidopsis*) reveals conserved domains. The structural features (length, ITRs size, 9 bp duplication) as well as the presence of conserved domains in the transposase strongly suggest that *Hop* is a *MuDR*-like element. This is the first active *MuDR*-like element found outside maize. Moreover, *Hop* excises from the *niaD* gene with a rather high frequency in spite of its location in an exon. This suggests that *Hop* is very active and thus represents a promising tool for developing an efficient insertional mutagenesis system.

189 **Expression and production of Llama variable heavy-chain antibody fragments (V_{HH}s) by *Aspergillus awamori*.** Vivi Joosten, Cees A.M.J.J van den Hondel and B.Christien Lokman. TNO Voeding, P.O. Box 360, 3700 AJ Zeist, the Netherlands.

High level production of antibody fragments is of industrial importance. Previous studies on the production of single chain (scFv) fragments in *Saccharomyces cerevisiae* demonstrated that these fragments accumulated in the Endoplasmic Reticulum (ER) and vacuole. It was postulated that the secretion of scFv might be hampered by improper folding of these fragments, or by the formation of large aggregates via interactions of the hydrophobic regions on the VH and VL domains (Frenken et al, 1994). Recent investments in *Aspergillus awamori* showed that scFv could be produced up to 200mg/l (Frenken et al, 1998). However, this production level is still too low for a cost effective large scale process. Serum of *Camelidae* (camels and llamas) contains IgG immunoglobulins that are devoid of light chains and therefore are referred as 'heavy-chain' antibodies. Since the variable domains of these heavy-chain antibodies (V_{HH}s) lack the hydrophobic regions that are normally facing the variable domain of the light chain, it is suggested that these fragments are better secreted than the scFv fragments. In *S. cerevisiae* antigen-specific V_{HH}s were secreted at levels over 100 mg/l in shake flask cultures. It is expected that the production level of V_{HH} by *A. awamori* will be even higher. In this study the production of four llama V_{HH}s (R2, R5, R7 and R9) raised against the azo-dye RR6, was analysed in *A. awamori*. Therefore, PCR fragments encoding these V_{HH}s were cloned in an Aspergillus expression vector containing the expression signals of the highly inducible endoxylanase gene. Northern analysis under inducing conditions revealed mRNAs of the expected size. Furthermore, Western analysis demonstrated that the four V_{HH}s are secreted in the culture medium. Intra- and extracellular protein levels were determined.

190 **Molecular genetic diversity of fungal communities in petroleum-contaminated soil using terminal restriction fragment patterns (TRFP).** N. S. Lord, P. Shank, L. Martinez, C. Reimers, K. Palmer, C. Kaplan, C. L. Kitts, S. L. Elrod. California Polytechnic State University, Biology, San Luis Obispo, CA, USA

Terminal restriction fragment pattern (TRFP) analysis was performed on DNA extracted from sand samples taken from a petroleum-contaminated pilot scale land treatment unit (LTU) on a dune system on the Central California Coast. The LTU consisted of three different treatment cells. Cell one was a control, cell two was amended with corn steep liquor, and cell three was amended with glucose. In addition, each cell was amended with nitrogen and phosphorous as well as aerated and hydrated on a regular basis. In order to assess temporal changes in fungal molecular genetic diversity during petroleum degradation, community sand DNA from each LTU cell was PCR amplified using primers to conserved regions of fungal 18S or ITS ribosomal regions; one primer was fluorescently-labeled. Amplicons were then digested separately with *Msp*I, *Hae*III, and *Dpn*II and terminal restriction fragments

(TRFs) were detected by capillary gel electrophoresis. Cells 1, 2 and 3 each returned unique TRFPs over the course of pilot-scale treatment. In addition, ITS TRFPs demonstrated a greater genetic richness (i.e. a larger number of peaks) than 18S TRFPs. These data suggest this TRFP method can be used to detect changes in the genetic diversity of fungal communities. Further analysis to identify the source of ITS TRFP richness (e.g. species or ribosomal gene copy number) is in progress.

191 **Characterisation of a gene involved in protein secretion in *Aspergillus niger.*** Peter J. Punt, Eric Record[a], Vivi Joosten and Cees A.M.J.J. van den Hondel. Department of Applied Microbiology and Gene Technology, TNO Nutrition and Food Research Institute Utrechtseweg 48, P.O. Box 360, 3700 AJ Zeist, The Netherlands, [a]Laboratoire de Biotechnologie des Champignons Filamenteux, Faculte des Sciences de Luminy INRA, CP 925, 163 Avenue de Luminy - 13288 Marseille Cedex 09, France

A major part of our research on filamentous fungi is dedicated to the characterization of the fungal secretion pathway. The ultimate aim of this research is to resolve bottlenecks for the efficient production of proteins in these organisms. Besides a more systematic approach, based on knowledge available from *S.cerevisiae*, in which specific secretion related genes were cloned and functionally characterized, we have also followed a more random approach based on selection of fungal mutant strains with perturbed secretion. Using a secretion reporter approach based on the *E.coli* glucuronidase (GUS) *A. niger* mutant strains were obtained with altered secretion characteristics. One of these mutants, GUS15.1#16, was unable to secrete the reporter protein, but also did not secrete significant amounts of glucoamylase. The strain also showed severely altered hyphal morphology, resulting in a hyper- branching phenotype. Using its restricted growth phenotype, the gene corresponding to the mutation was cloned by phenotypic complementation. For this purpose a cosmid library based on an autonomously replicating fungal transformation vector was generated and introduced in the mutant strains. Characterization of the complementing gene revealed that this gene showed significant homology with a sub-unit of a global transcriptional complex in *S. cerevisiae*.

192 **Production of proteins under the control of the trypsin-like promoter from *F. oxysporum* is not growth associated.** Natalie Farnworth, M.G.Wiebe, G.D.Robson and A.P.J.Trinci. School of Biological Sciences, University of Manchester, Manchester, M13 9PT.

A trypsin-like protease, with commercial value, has been identified in *Fusarium oxysporum*. The promoter from this protease has been used to drive the production of recombinant glucoamylase (GAM) in *Fusarium venenatum*. Trypsin-like protease was only produced in stationary phase in batch culture and the specific production rate did not increase with increasing specific growth rate. Similarly, native trypsin-like protease activity in *F. venenatum* was produced during stationary phase and was negatively correlated with growth rate. Further studies have shown that when GAM was produced in a *F. venenatum* transformant under control of the *F. oxysporum* trypsin-like protease promoter, it was only detected in culture supernatants after biomass increase (and therefore hyphal extension) had ceased. Western blot analysis suggested *de novo* synthesis of GAM occurred during stationary phase. This and other recombinant constructs thus provide a useful system for studying the production and secretion of proteins which are independent of hyphal tip extension.

193 **Characterization of beta-endoglucanase genes of industrial fungus *Aspergillus kawachii.*** Kiyoshi Ito, Yumi Hinoki, and Yukari Hara National Research Institute of Brewing, Genetic Engineering, Higashihirosima, Hiroshima, Japan

Filamentous fungus *Aspergillus kawachii* is a nearly related strain to *A. awamori* and it is widely used for shochu (a Japanese traditional spirit) fermentation. *A. kawachii* produces many kinds of cellulolytic enzymes along with sacchalolytic enzymes, so the enzymes from this strain can digest the cell wall of plants efficiently. We have cloned many genes of cellulolytic enzymes from *A. kawachii*. From them, we will present the characterization of three endoglucanase genes. EglA was an endoglucanase containing cellulose binding domain (CBD) at C-terminus with a very long linker region. The catalytic domain of EglA showed homology with family-5 endoglucanases. EglB had also CBD at C-terminus. The catalytic domain of EglB showed no homology with any cellulases but showed low homology with Cellulose-growth-specific protein (Cel1) from *Agaricus bisporus*. EglC showed high homology with the catalytic domain of EglA but had no CBD and linker. The transformed *Saccharomyces cerevisiae* with eglA, eglB, and eglC cDNA showed endoglucanase (CMCase) activity. The enzymatic properties were examined using the enzymes produced by yeast. EglA bound tightly to microcrystalline cellulose.

194 **Characterization of alpha-amylase genes of industrial fungus *Aspergillus kawachii.*** Toshikazu Sugimoto, Kenji Murashima, and Kiyoshi Ito. Kyowa Hakko Kogyo, Ibaraki, Japan. Natl Res Inst of Brewing, Hiroshima,

Japan Filamentous fungus *Aspergillus kawachii* is a nearly related strain to *A. awamori* and it is widely used for shochu (a Japanese traditional spirit) fermentation. Alpha-amylase is one of the most important enzymes in industrial process, so we examined the expression of alpha-amylase genes. *A. kawachii* produces two types of alpha-amylase, acid stable amylase (AA) and neutral amylase (NA). NA is almost the same as Taka-amylase (TA) of *A. oryzae*. NA was produced with glycerol or glucose as a carbon source, besides the common inducers such as starch or maltose. In the promoter region of NA gene, about 60 bp was depleted comparing the promoter of TA gene. Consequently, we considered that the constitutive expression of NA gene was due to the depletion of this region. AA was not produced in liquid culture but produced in solid-state culture. In liquid culture using maltose as a carbon source, strong signal of AA mRNA was detected by Northern analysis and strong GUS activity was detected by reporter gene analysis, in spite of the no AA activity. In this condition, no AA protein was detected inside and outside of the cell. Consequently, it was considered that the solid-state specific AA production was regulated at (post)- translational level.

195 **Control of hyphal morphology in *Aspergillus niger* by regulated expression of the *cotA* gene.** Shirley Burrow[1], Sarah Pollerman[1], Nigel Dunn-Coleman[2] and Geoffrey Turner[1]. [1]Department of Molecular Biology and Biotechnology, University of Sheffield, UK, [2]Genencor International, Palo Alto, CA

Strains of *Aspergillus niger* and the related species *A. awamori* are used in the commercial production of secreted native and heterologous enzymes. Limitations on enzyme yield in fermenter growth are influenced by mycelial morphology, which affects mycelial density, energy input and oxygenation. While mutations affecting morphology can arise during strain improvement programmes, the ability to manipulate morphology during fermenter growth by controlled expression of a specific gene would be advantageous, since an appropriate vector could be introduced into any strain of *A. niger* by transformation. In order to demonstrate the feasibility of this approach, we have cloned part of the *cotA* gene of *A. niger*, a homologue of the *Neurospora crassa cot-1* gene, and placed it under the control of the regulatable *glaA* promoter. The *cot-1* mutation of *Neurospora crassa* is a temperature sensitive mutation in a serine-threonine protein kinase, and growth at the non-permissive temperature leads to loss of polar growth of hyphae and to hyperbranching as a result of loss of function. Using a vector designed for promoter replacement, transformants of *A. niger* were selected on maltose as carbon source, and were screened for growth morphology on xylose. Approximately 60% of transformants showed a compact morphology on xylose, which represses *glaA*, and were purified for further analysis. These transformants also showed a compact growth form in xylose liquid medium in shake flasks, but were normal in maltose medium. These strains will be analysed for growth characteristics in fermenters.

196 **Structural features of maltose utilization gene clusters in *Aspergillus oryzae*.** Masahiro Takizawa[1], Takeshi Akao[2], Osamu Akita[2], and Katsuya Gomi[1]. [1]Graduate School of Agricultural Science, Tohoku University, Sendai, Japan. [2]National Research Institute of Brewing, Higashi-Hiroshima, Japan.

Aspergillus oryzae produces a copious amount of amylolytic enzymes such as a-amylase, glucoamylase, and a-glucosidase, which are most important in sake brewing. Recently, a transcriptional activator gene, *amyR*, involved in the amylolytic gene expression has been cloned (1). The *amyR* gene disruptants showed significantly poor growth on starch medium, but showed normal growth on maltose medium, indicating the existence of the maltose utilization enzymes, production of which might not be regulated by the *amyR*. An EST clone homologous to yeast maltase gene (*MAL62*) was found in the EST database constructed by *Aspergillus* EST sequencing consortium of Japan. Then two different gene clusters probably involved in maltose utilization have been isolated from an *A. oryzae* genomic library. One consists of *MAL62* homologue itself, designated *malT*, and a gene homologous to yeast maltose permease gene (*MAL61*), designated *malP*. In addition, a putative transcriptional regulator gene which has a typical fungal zinc finger motif at N-terminus is located at downstream of the *malT*, although this regulator gene, D*malR*, is unlikely functional because of truncation in the C terminal region. Another gene cluster is highly homologous to the sugar utilization gene cluster in *A. parasiticus* recently reported (2). Nucleotide sequence analysis of about 20-kb fragment encompassing the cluster revealed that the gene cluster has genes encoding a-glucosidase (*glcA*), a sugar transporter (*hxtA*), and a transcriptional activator (*sugR*) and is located at one end of the aflatoxin biosynthetic gene

cluster, as in *A. parasiticus*. (1)Gomi et al, *Biosci. Biotechnol. Biochem.*, **64**, 816-827 (2000). (2)Yu et al, *Biochim. Biophys. Acta*,

197 **Isoenzyme multiplicity and characterization of recombinant manganese peroxidases (rMnPs) from *Ceriporiopsis subvermispora* and *Phanerochaete chrysosporium*.** Luis F. Larrondo[1], Sergio Lobos[2], Phillip Stewart[3], Dan Cullen[3] and Rafael Vicuña[1]. [1] Departamento de Genetica Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Catolica de Chile, Santiago, Chile, and Millennium Institute for Fundamental and Applied Biology, Santiago, Chile. [2] Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile. [3] USDA Forest Products Laboratory, Madison, Wisconsin 53705.

We expressed cDNAs coding for manganese peroxidases from the basidiomycetes *Ceriporiopsis subvermispora* (MnP1) and *Phanerochaete chrysosporium* (H4) under the control of the a-amylase promoter from *Aspergillus oryzae* in *Aspergillus nidulans*. The recombinant proteins (Cs-rMnP1 and rH4) were expressed at similar levels and had molecular masses, both before and after deglycosylation, that were the same as those described for the MnPs isolated from the corresponding parental strains. IEF analysis of rH4 showed several isoforms with pIs between 4.83 and 4.06, one of which coincides with the pI (4.6) described for H4 isolated from *P. chrysosporium*. IEF of rMnP1 resolved four isoenzymes with pIs between 3.45 and 3.15, closely resembling the pattern observed with MnPs isolated from *C. subvermispora* grown on solid state cultures. The thermostability of both recombinant enzymes was also analyzed. We compared the rMnPs ability to use various substrates and found that rH4 could oxidize o-dianisidine and p-anisidine without externally added manganese, a property not previously reported for this MnP isoenzyme from *P. chrysosporium*. Acknowledgment: This work was financed by grants 8990004 and 2000076 from FONDECYT-Chile and by U.S. Dept. of Energy grant DE-FG02-87ER13712. L.F.L is a Predoctoral Fellow of Fundación Andes.

198 **Heterologous expression and secretion of an acetyl xylan esterase (AXE II) from *Penicillium purpurogenum* in *Saccharomyces cerevisiae* and *Aspergillus nidulans*.** Luis F. Larrondo, Francisco Herrera, Soledad Quiroz, Marcela Colombres, Alessandra Peirano and Jaime Eyzaguirre. Laboratorio de Bioquímica, Universidad Católica de Chile, Santiago, Chile.

P. purpurogenum secretes several esterases to the culture medium, among them AXE II. This enzyme was purified, sequenced and its three-dimensional structure determined by X-ray diffraction. Site-directed mutagenesis studies require a heterologous expression system. Here we describe the results obtained in the expression of the wild type enzyme in two different hosts. The coding region of the mature cDNA of AXE II was cloned in the yeast expression vector pBS24.1. The resultant plasmid (pFH5) was used to transform *Saccharomyces cerevisiae* AD3. Positive clones were selected and protein secretion was achieved by growing the transformants in YEPD with 2% glucose. Expression in *Aspergillus nidulans* was accomplished by fusing the cDNA of AXE II, including its signal peptide, with the *A. oryzae*Taka amylase promoter and the *A. awamori* terminator (pSQ1). Spheroplasts were co-transformed with pSQ1 and ppyrG, which confers prototrophy to uridine. Positive clones were grown in YEM with 2% maltose to induce the expression of the recombinant protein. rAXE II was followed by activity, western blots and SDS-PAGE in culture supernatants. *Saccharomyces* transformants secreted active enzyme, but several bands appeared in SDS-PAGE, while two bands of similar intensity were observed in western blots, one corresponding to the mature AXE II and another slightly larger, suggesting incomplete processing of the signal peptide. *Aspergillus* transformants secreted active enzyme and only one band identical in size to native AXE was observed in SDS PAGE and immunoblots. One of the selected clones produced up to18 mg/L of protein and 3.5 U/mg of AXE. In conclusion, *Aspergillus nidulans* is a good host for the expression of AXE II; rAXE II is secreted in an active form and in significant levels, permitting rapid detection and purification. This will facilitate the expression and characterization of site-directed mutants of AXE II. Acknowledgment: Work supported by DIPUC and by grants 8990004 and 2000076 from FONDECYT, Chile. L.F.L is a Predoctoral Fellow of Fundación Andes.

199 **Cloning the gene encoding acetyl xylan esterase from *Aspergillus ficuum* and its expression in *Pichia pastoris*.** Hea-Jong Chung, Seung-Moon Park*, Moon-Sik Yang and Dae-Hyuk Kim. Chonbuk National University, Biological Sciences, Chonju, Chonbuk, Korea. Chonbuk National University, *Basic Science Research, Chonju, Chonbuk, Korea

Acetyl esterases are important in the complete degradation of acetylated polysaccharides, such as pectins and xylans. We isolated the gene encoding acetyl xylan esterase (AfAXE) from a genomic library of *Aspergillus ficuum.* We cloned the corresponding cDNA by RT-PCR. The Afaxe gene contained two introns, one TATAA box, and two CAAT-like boxes. The transcription initiation site was 61 bp upstream of the start codon. The deduced amino acid sequence consisted of a putative 28-amino acid leader peptide and a mature protein with an estimated molecular mass of 29.5 kDa. The nearest homolog of the cloned gene was acetyl xylan esterase of *A. niger*. The cloned gene was placed in a Pichia expression vector and expressed in Pichia pastoris. The culture filtrate of the transformant liberated acetyl moieties from p-nitrophenyl acetate and its activity reached 75.8 IU/ml, which was over 100-fold greater than the activity of the native enzyme expressed in *A. ficuum*. The native and recombinant acetyl xylan esterases were purified from the culture filtrates of *A. ficuum* and *P. pastoris*, respectively. Both enzymes had approximately the same optimal temperature (37 C) and pH (7.0). The recombinant protein had greater tolerance for alkaline conditions (> pH 7.0), but was less thermostable above 55 C. The cloned enzyme catalyzed the release of acetic acid from acetylated hardwood xylan, confirming that the cloned gene encoded an acetyl xylan esterase of *A. ficuum*. This work was supported by 2000's Korea Research Foundation Grant.

200 **The foldase, CYPB, from *Aspergillus niger* contains a novel endoplasmic reticulum retention signal and its overexpression improves yields of secreted glucoamylase.** Patrick M.F. Derkx, Harm J. Mulder and Susan M. Madrid. Danisco Cultor Innovation, Langebrogade 1, DK 1001, Copenhagen, Denmark

Screening of *Aspergillus niger* libraries with degenerate oligo nucleotides encoding the endoplasmic reticulum (ER) retention signal HDEL, led to the isolation of the *cyp*B gene. The *cyp*B gene encodes a peptidyl prolyl *cis-trans* isomerase (PPIase) belonging to the cyclophilins and contains an ER targeting signal and a novel ER retention signal. The ER retention signal, HEEL was shown to be capable of retaining the green fluorescent protein (GFP) within the ER. The expression of *cyp*B was upregulated by the unfolded protein response and heat shock. *Escherichia coli* expressed and purified CYPB-$(His)_6$ is capable of isomerising a substrate peptide *in vitro*. The intracellular level of CYPB in *A. niger* was increased by introducing a plasmid allowing constitutive expression of *cyp*B. This enhanced the yield of native secreted glucoamylase 3 to 10 fold. Our results suggest that CYPB is a rate limiting step in the secretory pathway of *A. niger*. In the ongoing process of the improvement of *A. niger* as a production organism for homologous and heterologous proteins, the identification of bottlenecks in protein secretion is of considerable biotechnological interest.

201 **Development of ABC transporter mutants of *Aspergillus nidulans* as innovative tools in drug discovery.** Alan C. Andrade, Ciska Braam and Maarten A. De Waard. Wageningen University, Lab. of Phytopathology, Wageningen, The Netherlands

Antimicrobial resistance is a global problem that undoubtedly concerns modern society. The World Health Organization together with public health authorities worldwide, are already developing action plans with strategies and guidelines to combat this growing problem. Drug resistance is not a new phenomenon but has evolved since man began to use antibiotics. However, the development of new families of antimicrobials in previous decades made us believe that we could always remain ahead of the pathogens. Concern emerged in the late 1990s that resistance was accumulating without the discovery of new antimicrobials. A traditional way to discover these agents is broad-based whole cell screening with libraries of chemicals or natural extracts. A general problem facing this type of screening is that the ambient concentration of drugs is prone to be low. This problem is amplified by the function of multidrug pumps which occur in all living organisms. These pumps reduce the accumulation of drugs inside the test organism and will inevitably diminish the chances of the discovery of new drugs. Mutants of micro-organisms lacking multidrug-efflux pumps have significant potential for enhancement of the sensitivity to antimicrobial agents. Our major goal is to develop such mutants of *Aspergillus nidulans*. Using a combination of classical and molecular genetics, multiple knock-out mutants of ABC transporters (*atr*) genes from this fungus are being generated in different combinations. Results indicate that these mutants indeed display increased sensitivity to a broad range of toxicants. Hence, these mutants are usefull tools in screening programs of new lead compounds with antifungal activity.

202 *Chrysosporium lucknowense*, **a new fungal host for protein production.** Cora van Zeijl[1],Peter Punt[1], Mark Emalfarb[2], Rich Burlinghame[3], Arkady Sinitsyn[4], Martine Parriche[5], Jean-Christophe Bousson[5], and Cees van den Hondel[1]. [1]TNO Nutrition and Food Research Institute, Applied Microbiology and Gene Technology, Zeist, The

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Chrysosporium lucknowense, an ascomycetous fungus, not closely related to *Aspergillus* or *Trichoderma*, is developed as a new fungal host for protein production. This thermophilic fungus is able to secrete large amounts of (hemi)cellulases. Mutant strains of *C. lucknowense* have been isolated with 5-50 fold improved protein yields (per gram biomass) compared to available *Aspergillus* and *Trichoderma* strains. Large- scale fermentations were developed up to 150,000 litre cultures. Growth conditions of *Chrysosporium* strains are very versatile from acid to alkaline pH and at temperatures of 25-43 C. Several transformation systems have been developed for this fungus, based on nutritional or dominant selection markers. The use of this fungal system for the production of fungal and non- fungal proteins will be discussed.

203 **Seeing the light with quantum dots.** S.T. Merino[1], T. Prendergast[2], S. Nie[2] and M.E. Zolan[1]. Department of Biology[1] and Chemistry[2], Indiana University, Bloomington, Indiana

Our research focus is studying genes involved in DNA repair and meiosis in the fungus *Coprinus cinereus*. One commonly used assay is Fluorescence *in situ* Hybridization (FISH), which is used to examine pairing between homologs. Our lab is also using immunolocalization to examine protein localization in wildtype strains and meiotic mutants. Currently, we are attempting to modify these fluorescent techniques by using Quantum Dot technology. Quantum Dots (QDs) are cadmium selenide nanocrystals, capped with a layer of zinc sulphide. Luminescent QDs can be coupled to biological molecules. In comparison to organic dyes such as rhodamine, QDs are 20 times as bright, 100 times as stable, and one-third as wide in spectral linewidth. Using a streptavidin-conjugated QD, which will bind to biotinylated DNA probes, QDs can be used for FISH analysis. Using QD technology results in less background and brighter signal, which is especially useful in examining sister-chromatid cohesion. For immunolocalization, a QD is covalently coupled to an antibody, resulting in a fluorescent tag with stronger, specific signal. The potential use for Quantum Dot technology in fungal research appears unlimited.

204 **Analysis of the penicillin biosynthetic gene cluster: directed strain improvement of *Penicillium chrysogenum*.** Marco van den Berg, Richard Kerkman, Ilja Westerlaken, Chris Leeflang, Eric Koenhen and Roel Bovenberg. DSM Life Sciences, Division Anti-Infectives (624-0270), PO Box 1, 2600 MA Delft, The Netherlands.

Within the various business groups of the DSM Life Sciences Cluster research efforts are focussed on the development and improvement of microbial strains and production processes. Filamentous fungi like *Aspergillus niger* and *Penicillium chrysogenum* are two of our preferred organisms, the so-called pluGbugs, for enzyme and B-lactam antibiotics production, respectively. To optimize the production processes it is essential to have detailed knowledge on these organisms. For years we have for filled this with classical biochemical and genetical analyses of strains and fermentations. Recent additions to the molecular and biochemical toolkit rapidly increased the possibilities: genome sequencing, DNA arrays, proteomics, NMR, Green Fluorescent Protein, etc. In this presentation I want to present the most recent results obtained at our laboratories by applying these techniques to one of our production organisms: *Penicillium chrysogenum*. In previous studies we have shown that is possible to increase penicillin production of the laboratory strain Wisconsin54-1255, by transforming it with different combinations of the penicillin biosynthetic genes (1). A detailed analysis revealed a complex regulatory system (2). Recently, it was demonstrated that not only the penicillin biosynthetic enzymes determine the production rate, but regulatory circuits in primary metabolism (e.g. NADPH- balans) have a distinct influence (3). To get more insight in the processes involved we determined the complete sequence (60 kb) of the penicillin biosynthetic gene cluster. These gene cluster was shown to be amplified in industrial production strains (4). A detailed (functional) analysis of all ORFs will be discussed.

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205 **Characterisation of the translation initiation factors of *Trichoderma reesei.*** Anne Huuskonen[1], Edward Alatalo, Markku Saloheimo[1], Merja Penttil [1], and Joop van der Laan[2]. [1]VTT Biotechnology, P.O. Box 1500, 02044 VTT, Finland. [2]Genencor International B.V., P.O. 218, Leiden, The Netherlands

Protein translation is regulated in eukaryotes in response to various stress conditions. Inhibition of protein secretion leads to reduced translation initiation rates in mammalian cells. Our aim has been to characterise translation initiation factors of the filamentous fungus *Trichoderma reesei* both at gene and protein levels and to address possible feedback control between secretion and translation initiation in filamentous fungi. The genes encoding the translation initiation factors eIF2alpha, eIF2beta and eIF4A have been isolated and sequenced from *T. reesei*. Based on the results from the Southern hybridisation all three genes are present in the *Trichoderma* genome in one copy. The eIF2beta gene contains three introns which can be differentially spliced generating a population of mRNAs with different ORFs. The expression levels of the eIF genes have been examined in mycelia treated with different chemical agents known to have influence on protein translation and/or secretion. These agents include dithiotreitol (DTT) and Brefeldin A (BFA). Of the chemicals studied the reducing agent DTT appears to have the most drastic effect on all the three genes. DTT's effect is most pronounced in the case of the eIF2beta where the drug has been shown to alter the ratio of the different mRNA forms. The effects of the different drugs on the translation initiation factor protein levels has also been under investigation. To obtain more information on the effects of the eIF2alpha, one of the key regulatory factors in translation initiation, two different strains expressing a mutant form of this gene have been constructed. One strain is expressing the non-phosphorylative form of this protein and the other strain mimics the constitutively phosphorylated form of the protein. The mutations introduced to the gene appear to have only minor effects on the protein production and growth, possibly on the germination stage.

This study has been made within the Eurofung project funded by the EU.

206 **Cloning and relational analysis of 15 novel fungal endoglucanases from family 12 glycosyl hydrolase.** Frits Goedegebuur[1]*, Timothy Fowler** Jay Phillips#, Pim van der Kley[1], Piet van Solingen[1], Lydia Dankmeyer[1], and Scott D. Power[2]. [1]Genencor International B.V., Archimedesweg 30, 2333CN Leiden, The Netherlands. [2]Genencor International Inc., 925 Page Mill Road, Palo Alto, CA 94304-1013, U. S. A. # Current address: JPhillips@microcide.com ** Current address: tim@bainbridge.net

Cellulases belong to the large family of glycosyl hydrolases (GHs) and are produced by a variety of bacteria and fungi. These extracellular enzymes act as either endoglucanases (EGs) or cellobiohydrolases (CBHs). This poster describes molecular screening for EGs from the GH family 12. Using three homologous boxes deduced from 5 previously known members of the family we analysed cellulase producing fungal strains obtained from a diverse area of the fungal kingdom. Polymerase chain reactions (PCRs) using degenerate primers designed to the homologous protein boxes were used to identify the family 12 homologs. Fragments from these PCRs were used as templates for a second semi nested PCR. Gene specific primers were developed to determine the up and downstream sequences. Rapid Amplification of Genomic Ends (RAGE), a PCR based genome- walking technique was used to elucidate full-length gene sequences. Several fungi showed the presence of multiple versions of the gene while sequence analysis showed a diversity in 15 novel members of the family ranging from 27 to 94% similarity.

207 **Role of virulence proteins in *Agrobacterium tumefaciens*-mediated transformation of *Aspergillus awamori.*** C.B. Michielse, A.F.J. Ram, H. van Attikum, P. Bundock, P.J.J. Hooykaas, C.A.M.J.J. van den Hondel. Institute of Molecular Plant Sciences, Clusius Laboratory, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

Agrobacterium tumefaciens, a plant pathogen, is widely used for the transformation of plants, yeast and filamentous fungi (de Groot et al. 1998). *A. tumefaciens* transfers a part of its tumor inducing plasmid (T-DNA) to the host, where it integrates into the genome. The tumor inducing plasmid also contains a virulence region encoding proteins, which are involved in generation, transfer and integration of the T-DNA. The integration of T-DNA is a random process and therefore *Agrobacterium* can be used as a tagging system. The critical parameters involved in *Agrobacterium*-mediated transformation of *Aspergillus awamori* were identified and used to optimize the transformation protocol, resulting in a highly reproducible and efficient transformation system. To assess the role of *A. tumefaciens* virulence proteins on T-DNA transfer, several *A. tumefaciens* mutants were tested in their ability to transform *A. awamori*. These experiments showed that host range factors, like VirH and VirF, are not important for

A. awamori transformation. Mutation in the regulatory proteins (VirA, VirG), transport pore proteins (VirB) and proteins involved in generation of the T- strand (VirD1, VirC2) reduces or abolishes the formation of transformants.

1. de Groot et al. (1998) *Agrobacterium tumefaciens*-mediated transformation of filamentous fungi, Nature Biotechnology 16: 839-842.

208 **ER stress response: The *A. niger* transcription factor HacAp mediates the upregulation of the molecular chaperone *bip*A and the peptidyl prolyl cis-trans isomerase *cyp*B.** Harm J. Mulder, Patrick M. F. Derkx and Susan M. Madrid. Danisco Cultor Innovation, Langebrogade 1, DK 1001, Copenhagen, Denmark.

The accumulation of unfolded proteins within the lumen of the ER results in the activation of an intracellular signaling pathway leading from the ER to the nucleus. This signaling pathway is known as the Unfolded Protein Response (UPR) and results in an increased transcription of genes encoding ER resident proteins. One of the key elements in the UPR is HacAp, a bZIP family transcription factor that binds to the promoters of the target genes when the UPR is induced. The promoters of *bip*A and *cyp*B contain several putative UPR elements (UPRE) based on sequence similarity to the yeast and mammalian UPRE as well as a heat shock element. We have used transcriptional fusion between varying lengths of 5' upstream region and the *E.coli* GUS gene to delineate the unfolded response elements of the genes encoding the ER resident proteins: *cyp*B and *bip*A. In addition, gel mobility band shift assays were performed using purified HacA protein and the different putative UPREs. The regulation of the different UPR target genes (*bip*A, *cyp*B) was studied using different conditions of HacA overexpression. The transcriptional regulation of HacA was altered by fusing the hacA gene to a strong inducible glucoamylase promoter (GLA) and the constitutive GPDA promoter. The overexpressing HacA transformants were analysed by northern and western-immunoblot analyses.

209 **Aminopeptidase Y and carboxypeptidase Y of *Aspergillus niger* are targeted to the vacuole.** Basten E.J.W., Muller Y., Visser J., Schaap P.J.. Molecular Genetics of Industrial Microorganisms Wageningen University, Wageningen, The Netherlands

The fungal vacuole is thought to have several important physiological functions. Degradation is one obvious function but it is also a site for storage of metabolites. The vacuole is part of both the secretory and endocytic pathways and is also directly accessible from the cytosol. Most of the vacuolar enzymes are delivered to the vacuole via the early compartments of the secretory pathway and the endosome. In yeast several vacuolar proteases have been described. Most of these proteases are synthesized as inactive precursors that are transported to the vacuole. Since both, proteins that are secreted and proteins destined for the vacuole use the same parts of the secretory pathway, there must be signals within the proteins themselves that allows them to be sorted and targeted in a precise and efficient manner. After delivery to the vacuole the vacuolar proteases are matured, by removal of the propeptide, by the protease PrB. The propeptide also functions as an autoinhibitor. In the past vacuolar proteases of Aspergillus niger have been cloned and characterized, however little is known about maturation and vacuolar targeting of these proteases. To study in more detail, the maturation and vacuolar targeting of aminopeptidase Y in *Aspergillus niger*, a fusion construct was made with GFP. Results on the subcellular location and maturation of the (fusion) protein will be presented.

210 **Recombinant t-PA production in *Aspergillus niger* and *Trichoderma reesei.*** Karin Lanthaler, Atul Karandikar, Marilyn G. Wiebe, Geoff D. Robson and Anthony P. J. Trinci. University of Manchester, Microbiology, Manchester, Manchester, United Kingdom

Recombinant t-PA has been produced in two filamentous fungi, *Aspergillus niger* and *Trichoderma reesei*. t-PA production was studied in batch and chemostat cultures of *A. niger* and in chemostat cultures of *T. reesei*. The concentration of t-PA produced by *A. niger* was enhanced by addition of soya peptone to a defined medium. In batch cultures, 1.9 mg total t-PA [g biomass]$^{-1}$ was produced in this medium at pH 6.0, of which only 4.3 mg [g biomass]$^{-1}$ was active. Active and total t-PA were rapidly lost from culture supernatant during stationary phase. However, 2.7 mg total t-PA [g biomass]$^{-1}$ was produced in glucose-limited chemostat cultures (D = 0.07 h^{-1}, pH = 6.0, 25 C) for at least 140 h of continuous medium flow. t-PA could also be produced in chemostat cultures of the *Trichoderma reesei* transformant. Addition of soya peptone to the medium did not substantially increase the amount of t-PA

produced by this strain. In lactose-limited chemostat cultures 0.14 mg total t-PA [g biomass]$^{-1}$ was produced for up to 140 h of continuous medium flow.

211 **Characterisation of KexB, the kexin-like maturase of *Aspergillus niger*.** Ruud Jalving, Peter van de Vondervoort, Jaap Visser and Peter Schaap. Section Molecular Genetics of Industrial Microorganisms, Wageningen University and Research Centre, Wageningen, The Netherlands.

In filamentous fungi an effective method to enhance the yields of secreted foreign proteins is the use of a translational fusion between the target protein and an endogenous secreted carrier protein. Removal of the carrier protein is usually achieved in vivo through cleavage of an engineered KEX2 endoprotease recognition site at the fusion junction. We have cloned the kexin- encoding gene of *Aspergillus niger* (*kex*B). Disruption of *kex*B resulted in a transformant with a hyper-branching morphology. Using fluorogenic substrates kexin-like activity was measured in membrane-protein fractions of the wild type strain and a KexB overexpressing strain. In contrast, no kexin specific activity was detected in similar protein fractions of the *kex*B-disrupted strain. Expression in this loss of function strain of a glucoamylase human interleukin-6 fusion protein with an engineered KEX2 dibasic cleavage site at the fusion junction resulted in secretion of unprocessed fusion protein.

212 ***het*-gene homologs in filamentous fungi.** Theo van der Lee, Cees Waalwijk. Plant Research International, P.O. box 16, 6700 AA Wageningen, The Netherlands.

In filamentous fungi fusion of hyphae of the same individual generates a network of hyphae or mycelium. Fusion of somatic hyphae between genetically distinct individuals of the same species is usually restricted by vegetative incompatibility, a process of cell death and growth inhibition that occurs after fusion (reviewed in 1). This process is governed by *het*-genes and combinations of incompatible *het*-genes can be either allelic or non- allelic. The number of different functional alleles of *het*-gene loci is limited, but the number of these loci and their allele frequencies imply that under natural conditions, somatic hyphal fusion between different genotypes is rare (1). A number of *het*-genes have been cloned and they encode a variety of proteins. How these proteins trigger cell death and how *het*-genes evolved is unknown. The *het-c* gene of *Neurospora crassa* encodes a glycine rich protein that is probably located in the cell wall (2). Searches in both genomic (3) and EST databases revealed the presence of a homolog of *het-c* in *Neurospora crassa*. This homolog is distinct from the *het-c* alleles previously described. It lacks some highly variable regions but still has highly significant homology at the protein level. Searches in databases from other fungi and PCR with degenerate primers show homologs in a wide range of fungi. Interestingly, the homologs of other fungi show more homology to the newly found *het-c* homolog in *Neurospora crassa*, indicating that this may be the ancient gene from which the *het*-genes originated. Paralogs and probable orthologs of other het-genes were also found in filamentous fungi but not in other organisms like yeast, plants and animals, suggesting that these homologs are general only to filamentous fungi. The *het*-gene homologs may help to identify how these genes evolved and thus, might offer new insight in the process of vegetative incompatibility.
(1) Glass et al. (2000) Ann. Rev. Genet. 34:165-186 (2) Saupe et al. (1996) Genetics 143: 1589-1600
(3) http://www.mips.biochem.mpg.de/proj/neurospora

213 **Creation of a federated database for *Magnaporthe grisea* integrating genetic, physical, and BAC end sequence data.** Stan Martin, Sheila Dunn, Barbara Blackmon, T.D. Houfek, Rod Wing and Ralph A. Dean. Fungal Genomics Laboratory, North Carolina State University, Raleigh NC USA

We are in the process of sequencing the genome of *Magnaporthe grisea* , the causal agent of rice blast disease. This information will be useful for understanding the mechanisms of fungal infection and for developing strategies to counteract disease caused by this fungus. We have created a searchable database integrating end sequence data from BAC clones, genetic marker data, and contig assembly data that represent the majority of the *M. grisea* genome. A previously generated library of BAC clones representing a 25-fold coverage of the entire genome was end sequenced and fingerprinted by a *HindIII* digestion. The Image/FPC software package was then used to generate an assembly of ~188 contigs covering >95% of the genome. The database contains the results of this assembly and previously generated RFLP data. We used the RFLP data to physically anchor most of the FPC contigs onto one of the seven chromosomes in the *M. grisea* genome. We used AceDB for our core database engine and used the MySQL relational database, which we populated with numerical representations of sequence information, to create appropriately scaled images. The database will facilitate efforts to fill in the gaps in the parts of the genome that

remain to be sequenced. The database also allows researchers attempting to map known genes, or other sequences of interest, rapid and easy access to the fundamental organization of the *M. grisea* genome. Repetitive sequences such as MAGGY and Pot2, are prevalent within the genome, and may potentially undermine the fidelity of the contig assembly. In order to minimize the impact of this source of potential error, we have undertaken an extensive analysis of the distribution of these repeats.

214 **Diversification of heterologous DNA in Neurospora.** J.P. Rasmussen[1], P.J. Yeadon[1], F.J. Bowring[1], E. Cambereri[2], W.D. Stuart[2] and D.E.A. Catcheside[1]. [1] School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, South Australia 5001. [2] Neugenesis Corporation, 871 Industrial Road, San Carlos, California 94070.

Recombination hotspots active in meiosis provide a means of diversifying pairs of DNA sequences differing at multiple sites. We have constructed plasmids that permit targeted transfection of heterologous genes such that they are located between *his-3* and the *cog* hotspot in *Neurospora crassa*. This positioning enables enrichment for sequences which experience exchanges in the heterologous DNA during meiosis by selecting progeny that are recombinant at *his-3*. We have used this system to shuffle human immunoglobulin kappa chain sequences and also sequences from other fungi. Our system provides a novel method for accelerated evolution of genes in which new gene variants are expressed and the products secreted from cells without further manipulation.

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Host-Parasite Interactions

215 **Definition of tissue-specific and general requirements for plant infection in a phytopathogenic fungus.** Marie Dufresne, Andrew Foster and Anne Osbourn. Sainsbury Laboratory, John Innes Centre, Norwich, UK.

Surprisingly little is known about the factors that condition the ability of pathogens to colonize different plant tissues. *Magnaporthe grisea* has recently emerged as a paradigm for molecular genetic dissection of factors determining fungal pathogenicity to leaves, and mutational analyses have identified a number of genes required by *M. grisea* for pathogenic differentiation and colonisation of leaf tissue. We have shown that *M. grisea* can infect the roots of cereals, and that this fungus can be used for genetic analysis of factors determining root infection. Preliminary investigations using characterised *M. grisea* mutants from other laboratories have allowed us to identify mutants with leaf-specific, root-specific and general defects in ability to cause lesion formation on leaves and/or roots. For example, the melanin biosynthetic mutant *alb1* is non-pathogenic to barley leaves, but is unimpaired in its ability to cause lesions on roots of barley or wheat. Conversely, a global regulator of nitrogen assimilation (*NUT1*) is essential for root infection but is dispensable for disease development on leaves, while the MAP-kinase PMK1 and the predicted ABC transporter ABC1 are required for infection of both tissues and so represent general disease determinants. Screening of REMI mutants is now being carried out to identify novel determinants of tissue specificity and general factors required for colonisation of plants in *M. grisea*. To date two REMI mutants have been identified that fail to give lesions on wheat and barley roots. Characterisation of the genetic defects in these mutants is underway.

216 **A MAPK controls host root penetration and pathogenesis in *Fusarium oxysporum*.** Antonio Di Pietro, F. Isabel García-Maceira, Emese Méglecz, and M. Isabel G. Roncero. Departamento de Genética, Universidad de Córdoba, Spain.

The soilborne vascular wilt fungus *Fusarium oxysporum* infects a wide variety of plant species by directly penetrating roots, invading the cortex and colonizing the vascular tissue. A mitogen-activated protein kinase (MAPK), FMK1, controls several key steps in pathogenesis of *F. oxysporum*. Targeted mutants carrying an inactivated copy of *fmk1* show normal vegetative growth and conidiation in culture but are non-pathogenic on tomato plants. Conidia of *fmk1* mutants germinating in the tomato rhizosphere fail to differentiate penetration hyphae resulting in strongly reduced root attachment. Additional defects in *fmk1* mutants include impaired abilities to breach the liquid-air interface and to grow invasively on tomato fruit tissue. We are currently attempting to identify downstream effector genes whose expression is altered in *F. oxysporum* MAPK mutants. One such gene, *pl1*, shows drastically reduced transcript levels in *fmk1* mutants and encodes the cell wall-degrading enzyme pectate lyase.

217 **Functional analysis of two closely linked genes at the Tox1B Locus of *Cochliobolus heterostrophus.*** Mark Rose[2], Thipa Asvarak[1], Shun-Wen Lu[1], Xudong Zhu[1], Olen Yoder[1], GillianTurgeon[1]. [1]Department of Plant Pathology, Cornell University, Ithaca, NY 14853 [2]Novartis Agribusiness Biotechnology Research, Research Triangle Park, NC 27709

Cochliobolus heterostrophus race T, causal agent of Southern Corn Leaf Blight, requires T-toxin for high virulence on T-cytoplasm corn. Production of the polyketide T-toxin is controlled by two unlinked loci, *Tox1A* and *Tox1B* carried on 1.2 Mb of DNA not found in race O, a mildly pathogenic form of the fungus, or in any other *Cochliobolus* spp. or relative. Horizontal acquisition of the 1.2 Mb of DNA is suggested. PKS1, a polyketide synthetase encoded at *Tox1A*, has been proven necessary for T-toxin production. To identify genes at *Tox1B*, a race T cDNA library was successively probed, first with a *NotI* fragment from a *Tox1B* deletion mutant, then with the corresponding wild type fragment, to screen for genes missing from *Tox1B*. Closely linked decarboxylase (*DEC1*) and reductase (*RED1*) genes were identified. Disruption of *DEC1* in race T reduced both T-toxin production and virulence of the mutant on T- cytoplasm corn while disruption of *RED1* affects neither. Transformation of a large deletion mutant at *Tox1A* or at *Tox1B* with *PKS1* or *DEC1/RED1* respectively, or race O with all three genes, fails to restore toxin production, suggesting a cluster of genes necessary for toxin biosynthesis at both loci.

218 **A novel alcohol oxidase/RNA-binding protein with affinity for mycovirus double- stranded RNA from the plant pathogenic fungus *Helminthosporium (cochliobolus) victoriae.*** S. A. Ghabrial, Plant Pathology Dept., University of Kentucky, Lexington, KY 40546 USA

Diseased isolates of the plant pathogenic fungus *Helminthosporium (cochliobolus) victoriae* harbor two isometric dsRNA viruses. The diseased isolates produce little or no victorin, and are hypovirulent. We have cloned and sequenced a novel alcohol oxidase (Hv-p68) from *H. victoriae* that co- purifies with mycoviral dsRNAs. Hv-p68 was also detected as a minor component of the virus capsid. Sequence analysis revealed that Hv-p68 belongs to the large family of FAD-dependent GMC oxidoreductases and that it shares significant sequence identity (>67%) with the alcohol oxidases of the methylotrophic yeasts. Unlike the intronless alcohol oxidases from methylotrophic yeasts, a genomic fragment of the *Hv-p68* gene was found to contain four introns. Hv-p68, purified from fungal extracts, showed only limited methanol oxidizing activity and its expression was not induced in cultures supplemented with methanol as the sole carbon source. Northern hybridization analysis suggested that Hv-p68 expression is induced by virus infection since significantly higher Hv-p68 mRNA levels (10-20 fold) were detected in virus-infected isolates compared to virus- free ones. We demonstrated by gel mobility assays and Northwestern blot analysis that Hv-p68 exhibits RNA-binding activity and presented evidence that the RNA- binding domain is localized within the N-terminal region that contains a typical ADP-binding beta-alpha-beta-fold motif. In addition to RNA-binding activity, Hv- p68 also exhibits phosphotransferase/kinase activities, and is a candidate for the virion-associated kinase activity responsible for phosphorylation of the capsid protein. Hv-p68 is proposed to play a role in viral RNA packaging/ replication and in regulating viral pathogenesis.

219 **Cloning and characterisation of avirulence gene *Avr2* of *Cladosporium fulvum.*** Rianne Luderer, Frank L.W. Takken, Pierre J.G.M. de Wit and Matthieu H.A.J. Joosten. Laboratory of Phytopathology, Wageningen University and Research Centre, Binnenhaven 9, 6709 PD Wageningen, NL

The interaction between tomato and the biotrophic fungus *Cladosporium fulvum* complies with the gene-for-gene model. The tomato resistance locus *Cf-2* contains two homologous genes, *Cf-2.1* and *Cf-2.2*. Both genes confer a

hypersensitive response (HR)-mediated resistance to isolates of *C. fulvum* producing the matching elicitor. Attempts to clone the *Avr2* gene by reverse genetics have not been successful. Therefore, a PVX-based binary expression vector was used to allow *Agrobacterium tumefaciens*-delivered functional expression of a cDNA library of *C. fulvum* in tomato plants (Takken et al., Plant J., 24(2), 2000). Upon toothpick inoculation of *Agrobacterium* colonies onto tomato leaves, five independent clones, containing an identical open reading frame (ORF), were identified that gave *Cf-2*-specific HR. *Avr2* encodes a cysteine-rich protein of 78 amino acids (AA), with a predicted signal peptide for extracellular targeting of 20 AA. Tobacco lines expressing either *Cf-2.1* or *Cf-2.2* responded with a HR upon AVR2, indicating that both *Cf-2* genes confer AVR2 recognition. Strains of *C. fulvum* virulent on Cf2 tomato plants circumvent recognition by various single mutations in the ORF of the *Avr2* gene, that either result in a frameshift or in the insertion of a stopcodon. To prove that *Avr2* is indeed responsible for avirulence of *C. fulvum* on Cf2 plants, a strain virulent on Cf2 plants will be transformed with the *Avr2* gene.

220 **Sequence analysis and expression studies of *Erysiphe graminis* cDNAs from epiphytic mycelium.** S. Eckert[1], M. Both[1], A. DeBruijn[2], E. Mueller[2] and P. Spanu[1]. [1]Imperial College of Science Technology and Medicine, London, UK [2]Syngenta, Jealott's Hill Research Station, Berks, UK

The ascomycete fungus *Erysiphe graminis f.sp. hordei (Egh)* is an obligate biotrophic parasite which is the causal agent of barley powdery mildew. We have extracted RNA from epiphytic *Egh* mycelium 3 days after infection i.e.prior to "en masse" sporulation. This RNA was then used to construct a cDNA library in the phagemid vector lambdaTriplEx2. We have analysed the sequences of about 450 randomly selected clones (Expressed Sequence Tags -ESTs). From the pool of sequences analysed, 349 clones (78%) are over 200 nt. Of these sequences, 191 identify homologs with significant BLAST matches, of which 167 have known functions. About half of the sequences analysed are unique. Of the sequences over 200 bp, the most abundant group with known or deduced function are transposons and retroelements, followed by ESTs encoding proteins involved in translation, and those involved in signalling. The library also contains ESTs encoding deduced proteins ("hypothetical proteins") with similarity to 15 different unknown ORFs in other organisms. ESTs with no known homologies are 45%. We also present a comparison of the results with *Egh* ESTs from a library of germinating spores (Oliver et al. www/crc.dk/phys/blumeria). A few clones were selected and used to probe RNA blots to assess expression during different stages of development and infection (spores, pre-sporulating epiphytic mycelium and infected barley leaves with heavily sporulating colonies). The results also enable us to assess whether the abundance of these clones in the cDNA library reflects the level of expression in the mycelium. This study is the starting point for a global expression study on a *Egh* using micro-arrays.

221 **Activation of a plant secondary metabolite by *Botrytis cinerea*: Resveratrol acts as a profungicide.** Alexander Schouten, Lia Wagemakers, Francesca L. Stefanato, Rachel M. van der Kaaij & Jan A.L. van Kan. Department of Phytopathology, Wageningen University, PO Box 8125, 6700 EE Wageningen, The Netherlands.

In grape (*Vitis*) the secondary metabolite resveratrol is considered a phytoalexin, which protects it from *Botrytis cinerea* infection. Laccase activity displayed by the fungus is assumed to detoxify resveratrol and to facilitate colonisation of grape. We initiated a functional molecular-genetic analysis of *B. cinerea* laccases by characterising laccase gene(s) and evaluating the phenotype of targeted gene replacement mutants. Two different laccase genes from *B. cinerea* were characterised, *Bclcc*1 and *Bclcc*2. Only *Bclcc2* was strongly induced in liquid cultures by either resveratrol or tannins. This suggested that *Bclcc*2, but not *Bclcc*1 plays an active role in the oxidation of both resveratrol and tannins. Analysis of both *Bclcc*1 and *Bclcc*2 replacement mutants confirmed this. Only *Bclcc*2 replacement mutants were not capable of converting both resveratrol and tannins. When growing on resveratrol, both the wild type and the *Bclcc*1 mutant showed an inhibited growth and *Bclcc*2 replacement mutants were unaffected. Thus, contrary to the current theory, BCLCC2 does not detoxify resveratrol but converts it into compounds that are more toxic for the fungus itself. Consquently, rather than a virulence factor BCLCC2 can be considered an avirulence factor. The activation of a plant secondary metabolite by a pathogen introduces a new dimension to plant-pathogen interactions and the phytoalexin concept.

222 **Molecular identification of a sexual interloper: *Venturia pirina*, reproduces sexually on its nonhost, apple**. Kim M. Plummer[1], Christiane Stehmann[2] and Shaun Pennycook[2]. [1]The University of Auckland, School of Biological Sciences, Private Bag 92019, Auckland, New Zealand. [2]HortResearch, Mt Albert Research Centre, 120 Mt Albert Rd, Private Bag 92169, Auckland, New Zealand

Venturia inaequalis causes apple black spot (apple scab), the most economically important disease of apple world wide. *Venturia* spp. are pathogens on Rosaceae and are commonly identified on the basis of morphological characters and host specificity. Morphological characters are sometimes absent or ambiguous and so DNA sequence analysis was used to aid identification. rDNA sequence variations, between different *Venturia* spp., were utilised to design species-specific primers. rDNA sequencing was used to identify *Venturia* spp. from apple leaf litter in New Zealand (spring, 1998). *Venturia pirina* (pear scab pathogen) and *Venturia inaequalis* (apple scab pathogen) were detected as ascospores discharged from apple leaf litter. Pseudothecia of both species were located on the dead apple leaves, however only those of *V. inaequalis* were associated with scab lesions. *V. pirina* was identified using rDNA sequence analyses, as morphological characters could not distinguish this fungus from *V. asperata* (a rare saprophyte on apple) and other *Venturia* spp. pathogenic on rosaceous fruit trees. *V. pirina* field isolates were pathogenic on pear, but only weak saprophytes on apple. In rare instances, when appressoria of *V. pirina* appeared to penetrate the cuticle of apple leaves, epidermal cells responded with a localised hypersensitive response (HR). This is the first report of HR-like events induced by *V. pirina* on its nonhost, apple. This is also the first record of sexual reproduction of *V. pirina* on apple. It is assumed that *V. pirina* pseudothecia formed in senescing tissue, when defence mechanisms such as HR were no longer active.

223 **Genome organization at the site of integration for a horizontally transmitted element in a *Leptosphaeria* species.** Janet L. Taylor NRC Plant Biotechnology Institute, Saskatoon, SK Canada

Several *Leptosphaeria* species cause disease on cruciferous plants. These species are distinguished by the phytotoxins that they produce and their hosts. One of these *Leptosphaeria* species makes polyketide toxins and attacks Japanese horseradish. However, we found two isolates of this species that have a repetitive element from the related species, *L. maculans*. These two isolates have an expanded host range, now attacking brown and yellow mustard. Their metabolite profiles have changed with no production of polyketide toxins. Now they make a host-selective toxin that is a tricyclic sesquiterpene. We have isolated two cosmid clones covering the region of element insertion. Initial sequence analysis by blast search has revealed the following similarities to known proteins. An area with high similarity to a phosphatidylinositol glycan class O protein of mouse. These proteins are involved in transfer of phosphoethanolamine to the GPI anchors that attach proteins to the cell surface membrane. Another area has low similarity to reverse transcriptase genes found in retrotransposons. This sequence is separated from the inserted repetitive element, LMR1. Most interestingly, we have sequence that translates to a protein with a conserved motif of sesquiterpene synthases. This motif is the only region of significant similarity among the synthases. Further details of the genome organization will be presented.

224 **Biocontrol of *Fusarium oxysporum* infection via protoplast fusants possessing genes producing disease-suppressive agents.** Kamel A. Ahmed (1), Mohamed H. Hamoda (1), Mohamed S. Abd Elsalam (2) and Ola I. El-Hamshary (2) 1- Genetics Dept., Fac. Agric., Cairo Univ. 2- Microbial Genetics Dept., NRC., Egypt.

The capacity of some microbial strains to produce biocontrol agents such as antimicrobial substances and iron chelates was determined. Pair-wise protoplastfusants between these strains e.g. *Pseudomonas fluorescens*, *P. aurogenosa*, *Entrobacter cloacae* and *Trichoderma harzianum* were carried out. Efficiency of these fusants on infectivity of *Fusarium oxyspoum* will be discussed.

225 **Xylanases of *Magnaporthe grisea*: differential requirement for pathogenicity**. Shengcheng Wu, Zhiying Zhao, Alan G Darvill, and Peter Albersheim. University of Georgia, Complex Carbohydrate Research Center, Athens, Georgia, USA

Phytopathogenic fungi that infect Gramineae tend to secrete copious amount of arabinoxylan-degrading enzymes. These enzymes are proposed to be pathogenic factors for they help in breaking down the cell wall barrier and obtaining nutrients so that the fungi can enter and flourish inside plants. *Magnaporthe grisea*, the rice blast fungus, produces multiple isoforms of endo-beta-1,4-D-xylanases under various growing conditions. We have cloned a total of six xylanase genes that are differentially expressed both in fungal culture and in infected rice seedlings. For example, two (*Xyl4* and *Xyl5*) are expressed exclusively in infected rice leaves. Infection assays of knockout mutants indicate significant reduction in pathogenicity of the mutants that lack anyone of *Xyl3*, *Xyl4* and *Xyl5* genes. Additional multiple knockout mutants (*xyl3xyl4*, *xyl3xyl5*, *xyl4xyl5* and *xyl3xyl4xyl5*) will be generated, and their phenotype and pathogenicity will be examined and presented. Interestingly, careful infection analysis of the

previously described *xyl2* mutants on various ages of rice seedlings reveals convincing increase in virulence. Thus, it is possible that either Xyl2 or the hydrolytic products of Xyl2 is recognized by rice as elicitor. (This work was supported by U. S. Department of Energy grant DE-FG05-93ER20114 and the DOE-funded (DE-FG05-93ER20097) Center for Plant and Microbial Complex Carbohydrates.)

226 **Cross-infection and survival of *Colletotrichum acutatum* from strawberry in strawberry and nonspecific hosts using transformants containing GFP constructs.** S. Freeman[1], S. Horowitz [1,2], and A. Sharon [3]. [1]ARO, The Volcani Center, Bet Dagan 50250, and [2]Tel Aviv University, Tel Aviv 69978, Israel

Colletotrichum acutatum, the cause of strawberry anthracnose, is one of the most important fungal pathogens of this crop worldwide. Cross-infection from additional hosts may exist since many botanical species are hosts to the pathogen and can serve as a source for primary inoculum. Host range, specificity and survival of *C. acutatum* from strawberry was examined with pepper, eggplant, tomato, bean and strawberry. Colonization and survival was confirmed by PCR-specific primer amplification for *C. acutatum*. Elucidation of the mode of infection and colonization of the pathogen in host and nonhost tissues was achieved using transgenic isolates. Transformants of *C. acutatum*, expressing the *UidA* (GUS) or *SGFP-TYG* reporter genes were obtained by electroporation with constructs containing a homologous DNA fragment. Major differences between pathogen interaction with the host and nonhost were observed 50hr post-inoculation. In strawberry, highly asymmetric appressoria developed due the formation of a penetration pore compared to symmetric structures in nonhost tissues lacking the pore. The strategy of invasion in strawberry included a short biotrophic phase in which the hyphae were mainly restricted to the intercellular space between the epidermal cells. Four days after symptom development, a large amount of intercellular and intracellular fluorescence accompanied by hyphal growth were observed with the GFP transformants. This marked the necrotrophic phase responsible for anthracnose and blight symptoms. While no visible disease symptoms were observed in the nonhost plants, extensive hyphal growth and appressoria were present on the leaf surfaces but were restricted to the intercellular spaces. Whether this pathogen can adapt as an epiphyte or possess an asymptomatic endophytic lifestyle in nonhost plants should be considered.

227 **Identification of gene products involved in plant pathogen interactions using proteomics.** Fitzgerald, A.M.[2], Stehmann, C.[1], Greenwood, D.R.[1], Plummer, K.M.[2] . [1] HortResearch, Mt Albert Research Centre, Private Bag 92 169, Auckland, NZ. [2] School of Biological Sciences, Auckland University, Private Bag 92 019, Auckland, NZ

Apple scab is caused by the ascomycete fungus *Venturia inaequalis.* In contrast to many fungal plant pathogens, *V. inaequalis* grows between the cuticle and epidermal cells during its biotrophic phase of growth. At this stage, the pathogen does not ingress into or disrupt plant cells. Consequently, the intercellular space (apoplast) is the plant/pathogen interface and should contain plant and pathogen gene products that are responsible for the maintenance and specificity of the disease interaction.

The aim of this project is to identify the fungal proteins that confer avirulence and pathogenicity in addition to plant disease response proteins from apoplastic fluids of scab infected apple. These apoplastic fluids contain only a limited number of proteins and about 30-40 bands are visible on coomassie stained SDS-PAGE. Therefore, proteomics is a realistic approach to identify the proteins secreted into the apoplast during infection.

Proteins have been extracted from the apoplast of infected and uninfected apple seedlings. Protein extracts were heavily contaminated with phenolics and other low MW contaminants and methods had to be developed to remove these for successful two-dimensional (2D) electrophoresis. 2D electrophoresis separated approximately 200 apoplastic proteins of healthy seedling leaves, which are mainly acidic and of relatively low MW (20-40 kD). In infected leaves, apoplastic proteins appeared to be degraded to peptides of MW 3-6 kD. Therefore, it is not possible to identify additional secreted plant and/or fungal proteins, specific to this disease, by comparison of infected *vs.* uninfected tissue. The mechanisms involved in this proteolytic breakdown are under current investigation. Apoplastic proteins of infected and uninfected tissue are being analysed using MALDI TOF, ESI-MS/MS and Edman chemistry.

228 **ATP-binding cassette (ABC) transporters from *Botrytis cinerea*.** Maarten A. De Waard[1], Keisuke Hayashi[2], Henk-jan Schoonbeek[1], Tycho Vermeulen[1]. [1]Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands. [2]Ube Research Laboratory, Ube, Yamaguchi, Japan.

Botrytis cinerea is a pathogen with a broad host range. Host plants are known to produce plant defense compounds, implying that the pathogen is exposed to a variety of fungitoxic compounds. Obviously, the pathogen developed mechanisms to cope with plant defense compounds during evolution. One mechanism can be activity of ABC transporters, which reduce the accumulation of toxic compounds in mycelium. These transporters may also account for development of resistance to fungicides used to control diseases incited by the pathogen. To test these hypotheses, we have studied ten ABC genes (*Bcatr*A-N) from *B. cinerea*. Expression of *BcatrB* is upregulated by the grapevine phytoalexin resveratrol. *BcatrB* replacement mutants show an increased sensitivity to resveratrol and are less virulent on grapevine leaves. The same mutants have an increased sensitivity to phenazine antibiotics produced by *Pseudomonas* and the phenylpyrrole fungicide fludioxonil. These results demonstrate that BcatrB provides protection against natural toxic compounds and, at the same time, is a determinant in fungicide activity. Fungicides from different chemical classes not only induce transcription of *BcatrB* but of other *Bcatr* genes as well, suggesting that several ABC transporters effect sensitivity of *B. cinerea* to these products. Laboratory-generated mutants of *B. cinerea* resistant to azole fungicides display a decreased accumulation of azoles and an increased basal level of *BcatrD* expression. Transcript levels induced by azoles in azole-resistant mutants correlate with resistance levels as well. Therefore, we propose that BcatrD is the major ABC transporter involved in activity of azoles. Currently, we test this by phenotyping the sensitivity of disruption and overexpression mutants of *BcatrD* to azoles.

229 **ATP-binding cassette (ABC) transporters from the wheat pathogen *Mycosphaerella graminicola*.** Maarten A. De Waard, Marco M.C. Gielkens, Stephen D. Goodall, Ioannis Stergiopoulos, Koen Venema, and Lute H. Zwiers. Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands.

The fungus *Mycosphaerella graminicola* (anamorph state: *Septoria tritici*) is the causal agent of septoria tritici leaf blotch, one of the most important diseases of wheat. Little is known about mechanisms of pathogenesis and fungicide resistance in this pathogen. ABC transporters may play a role in these processes by providing protection against plant defense compounds and fungicides, respectively, or by secretion of virulence and mating type factors. This hypothesis forms the basis of our research. Five single copy genes encoding ABC transporters, designated *Mgatr1-5*, have been characterized. All genes have the [NBF-TMD6]2 configuration and are highly homologous to other fungal and yeast ABC transporters. Northern blot analysis revealed that they display distinct expression profiles when treated with secondary plant metabolites and fungicides. Expression of these genes was different in mycelium and yeast-like spores of this dimorphic fungus. Heterologous expression of the *Mgatr* genes in a strain of *Saccharomyces cerevisiae* with multiple disrupted ABC genes showed that the encoded proteins of most *Mgatr* genes can transport a range of chemically unrelated compounds, such as fungicides, mycotoxins, and secondary plant metabolites. The function of the *Mgatr* genes is also studied in disruption mutants of *M. graminicola*. These mutants were made by *Agrobacterium tumefaciens*-mediated transformation. We have developed this technique as an efficient tool to generate targeted gene disruption. At present, we are studying the phenotype of the disruption mutants with respect to sensitivity to toxicants and virulence.

230 **A MAP kinase module regulating filamentous growth, mating and pathogenic development in *Ustilago maydis.*** Philip Mueller, Michael Feldbruegge and Regine Kahmann. Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse D- 35043 Marburg, Germany

In the phytopathogenic fungus *Ustilago maydis* fusion of compatible haploid cells is a prerequisite for infection. This process is genetically controlled by the biallelic *a* locus encoding pheromone precursors and receptors. Binding of pheromone to its cognate receptor triggers the pheromone response characterized by conjugation tube formation and pheromone-responsive gene expression. It was proposed that the pheromone signal is transmitted via a MAP kinase cascade to the transcription factor Prf1. Three putative components of this MAP kinase cascade have been described, *kpp2 (ubc2)*encoding a MAP kinase, *fuz7* encoding a MAPK kinase and *kpp4 (ubc4)*encoding a MAPKK kinase. Deletion of either *kpp2*,*fuz7* or *kpp4* results in loss of conjugation tube formation and attenuates mating. While *fuz7* and *kpp4*deletion mutants exhibit a complete block in pathogenic development, *deltakpp2* mutants retain residual pathogenic potential. To analyze the connection between these signaling components in more detail we have generated constitutively active alleles of *fuz7 (fuz7DD)* and *kpp4 (kpp4-2).* Expression of *kpp4-2* leads to

elevated pheromone gene transcription and to conjugation tube formation in wild type cells, but not in *deltakpp2* or in *deltafuz7* strains suggesting that Kpp4 acts upstream of Fuz7 and Kpp2. Consistently, *fuz7DD* induces the same phenotype in a *kpp2*- dependent, but *kpp4*-independent manner. In addition we show that *prf1* is dispensable for this morphological transition. We conclude that Kpp4, Fuz7 and Kpp2 are members of one MAPK cascade that regulates Prf1 and yet unidentified target proteins triggering morphological changes.

231 **A gene directly regulated by the *b* mating type locus of *Ustilago maydis* is involved in pathogenic development.** Gerhard Weinzierl[1], Ralph Bohlmann[2], Andreas Brachmann[3], Tobias Hindemitt[1], Regine Kahmann[1] and Joerg Kaemper[1]. [1]Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch- Str., D-35043 Marburg, [2]Aventis Crop Science, 14 -20 Rue Pierre Baizet, B.P. 9163, F-69009 Lyon Cedex 09, [3]Institute of Genetics and Microbiology, Ludwig-Maximilians University, Maria-Ward-Str. 1a, D-80638 Muenchen

In *Ustilago maydis*, the multiallelic *b* mating type locus provides the central regulatory instance for the establishment of the pathogenic stage during its life cycle. This developmental control is achieved via the *b*-encoded bE and bW homeodomain proteins. The bE and bW proteins can form a heterodimer that is thought to function as transcriptional regulator for genes that play a crucial role during differentiation processes linked to pathogenicity. To get an insight into these processes, our aim was to isolate direct target genes of the bW/bE heterodimeric complex. Using differential display we have isolated the b-dependently expressed gene *dik6*, encoding a putative seven transmembrane receptor. The promoter elements essential for b-mediated regulation were identified by deletion analysis. Subsequent electrophoretic mobility shift experiments and DNAse protection assays using a bE-bW fusion protein substantiated that *dik6* is a direct target gene of the bE/bW complex. Deletion mutants of *dik6* show only an attenuation of pathogenicity, i.e. 36% of the wild type infection rate. However, the search of the *U. maydis* genomic sequence revealed another potential transmembrane receptor with 36% identity to Dik6. Interestingly, the respective gene, *dkh6*, exhibits a b- dependent expression pattern identical to *dik6*, suggesting for both genes a possible function during the pathogenic development. We will discuss these results with respect to potential functions of Dik6 and Dkh6 during the infection process.

232 **Genetics and ecology of fumonisin toxins.** Anne E. Desjardins, Ronald D. Plattner, Gary P. Munkvold, and Robert H. Proctor. USDA/ARS, National Center for Agricultural Utilization Research, Peoria, IL and Department of Plant Pathology, Iowa State University, Ames, IA

Fumonisins are a family of toxic fungal metabolites produced by *Fusarium verticillioides* (synonyms *F. moniliforme* and *Gibberella fujikuroi* mating population A) and related species. *F. verticillioides* is not only the most common fungal pathogen of maize, but also is among the most common fungi found in symptomless maize plants. The recent cloning of fumonisin biosynthetic genes and development of techniques for gene disruption now allow critical analysis of the importance of fumonisins in fungal infection and disease. A polyketide synthase gene, designated *FUM5*, that is required for fumonisin biosynthesis has been cloned from *F. verticillioides*. Disruption of *FUM5* in fumonisin-producing strains reduced fumonisin production by more than 99%. Two fumonisin- nonproducing (*FUM5*-) strains that were generated by *FUM5* disruption and the two fumonisin-producing (*FUM5*+) strains from which they were derived were tested for their ability to infect maize ears and to cause ear rot following four application methods. In field tests in Illinois and Iowa in 1999 and in Illinois in 2000, fungal spores were applied to ears by silk-channel injection and by silk-spray, to stalks by injection, and to seeds by planting next to a fungal-infested toothpick. Disease severity on harvested ears was evaluated by visible symptoms and by the relative weight of symptomatic and nonsymptomatic kernels. Fumonisin levels in kernels were determined by HPLC. Presence of applied strains in kernels was determined by analysis of recovered strains for fumonisin production by HPLC and for *FUM5* alleles by PCR. *FUM5*- strains were able to infect ears following all application methods. Following silk-channel application, *FUM5*- strains infected the kernels, caused ear rot, and inhibited the accumulation of fumonisins in the kernels. Application of *FUM5*- strains by silk-spray, stalk injection, or seed treatment increased the frequency of these strains in kernels, but did not increase levels of ear rot or affect the concentrations of fumonisins in the kernels. These results show that production of fumonisins is not required for *F. verticillioides* to cause ear rot following silk-channel injection, or to infect maize ears following silk-spray inoculation, stalk injection, or seed treatment. Thus, the importance of fumonisins in the ecology of *F. verticilliodes* remains unclear.

233 **Identification and characterization of pathogenicity mutants of *Colletotrichum lagenarium* by insertional mutagenesis**. Naoyuki Takayanagi[1], Yoshitaka Takano[2], and Tetsuro Okuno[1]. [1]Kyoto University, Graduate School of Agriculture, Kyoto, Japan. [2]NC State University, Plant Pathology, Raleigh NC USA

Colletotrichum lagenarium is the causal agent of anthracnose of cucumber. We have initiated a mutational analysis of fungal pathogenicity in *C. lagenarium* using restriction enzyme-mediated integration (REMI) mutagenesis. Here we present characterization of three pathogenicity mutants of *C. lagenarium*. Mutants KE51 and KH190 showed normal growth, conidiaion, germination, and appressorium formation. However, melanized appressoria formed by both mutants failed to penetrate into the host epidermal cells although they were able to penetrate cellulose membranes, which suggested that genes required for penetration into the host plant were impaired in these mutants. Mutant KH151 showed reduction in both growth and conidiation on nutrient agar, and failed to form appressoria. Furthermore, KH151 formed no lesions on cucumber leaves even when inoculated through wounded sites, suggesting lack of an ability to grow invasively inside the plant. Genomic DNA flanking the integrated plasmid was recovered from the mutant KE51. Using the recovered DNA as a probe, a genomic clone of the wild-type strain was isolated. The 4 kb *Hin*dIII fragment corresponding to the tagged site complemented the pathogenicity of KE51. These results demonstrate that this fragment contains a pathogenicity gene impaired in KE51. We determined DNA sequences of the plasmid insertion region in the complementing fragment, and found a single open reading frame that exhibited homology to genes encoding a methyltransferase-like protein identified in several organisms.

234 **Effects of double-stranded RNA on protease production and virulence of *Metarhizium flavoviride* against the grasshopper *Rhammatocerus schistocercoides* .** Maria Helena P. Fungaro[1], Mayra Kassawara Martins[1], Patrícia Vieira Tiago[1], Marcos Rodrigues Faria[2], Márcia Cristina Furlaneto[1].[1]Universidade Estadual de Londrina, Londrina, Pr, Brazil. [2]Cenargen, Embrapa, Bras lia, DF, Brazil

Bands of dsRNA were detected in five out of seven isolates of the entomopathogenic fungus *Metarhizium flavoviride*. In order to study the effects of dsRNA on protease production and virulence against the grasshopper, many methods were applied as attempts to obtain isogenic strains, differing by the presence or absence of dsRNA. Attempts to obtain isogenic strains by curing were unsuccessful. An alternative approach, i.e. the transfer of dsRNA via forced heterokaryosis was successfully conducted. The nuclear contents of the recipient colonies were confirmed to be identical to the parental strain by RAPD. The production of proteases determines virulence towards their hosts. So, the wild and isogenic strains (CG422 *met* and CG442 *met* dsRNA, without and with dsRNA, respectively) were analysed for Pr1 production and virulence against grasshopper Rhammatocerus schistocercoides. The results of Pr1 analyses showed no statistical differences among isolates with and without dsRNA. Data from bioassays also showed no statistical differences among isolates. We conclude that these dsRNA fragments do not cause hypovirulence to *M. flavoviride* .

235 **Genetic variation in the cuticle-degrading protease activity of the entomopathogen *Metarhizium flavoviride*.** Furlaneto, Marcia Cristina; Fungaro, Maria Helena Pelegrinelli; Pinto, Fabiana Gisele da Silva; Tiago, Patricia Vieira. University of Londrina, Microbiology, Londrina, Parana, Brazil

Extracellular proteases have been shown to be virulence factors in fungal entomopathogenicity. We examined the production of the cuticle-degrading extracellular proteases, subtilisin-like (Pr1) and trypsin-like (Pr2) in isolates of the fungus *Metarhizium flavoviride*. Fungal growth was in mineral-medium (MM) containing nitrate, and in MM supplemented with either cuticle from *Rhammatocerus schistocercoides* or with the non-cuticular substrate casein. The substrates used for growth influenced the expression of both proteases analyzed, and for nearly all isolates, the highest protease activities were observed in medium containing insect cuticle, with more Pr1 being produced than Pr2. There was natural variability in the production of cuticle-degrading proteases among isolates, although this was less evident for Pr2.

236 **The compatible interaction between potato and *Phytophthora infestans.*** Katinka Beyer[1], Andres Binder[2], Thomas Boller[3], and Margaret A Collinge[4]. [1]Friedrich Miescher-Institut, Basel, Switzerland. [2]Syngenta, Basel Switzerland. [3]Botanical Institut, Basel Switzerland . [4]University of Zuerich, Plant Biology, Zuerich Switzerland

Phytophthora infestans causes late blight disease on potato and tomato, which is the most important disease of potato worldwide. *P. infestans* is notorious for causing the Irish Potato Famine shortly after it first appeared in Europe in the 1840s. Despite the fact that it is known since a long time, we still know little about the molecular processes involved in infection and resistance. In this work we are examining the interaction between potato and *P.infestans* with a genomics approach, looking at the genes induced during infection in both, host and pathogen. To screen for induced genes we used suppression subtractive hybridization (SSH), comparing different stages of the infection process. Screening of the subtracted libraries is still in progress, but we have already isolated a number of potato genes and a few *P. infestans* genes. For a subset of the plant genes differential expression has been confirmed on Northern Blots. Some of the plant genes have not been reported to be pathogen induced before. In contrast to many other studies, we are concentrating on the compatible interaction. The induced genes are interesting at both the scientific and the biotechnological level. The plant genes may include classic PR genes or genes involved in tolerance and in late-expressed resistance. The *P.infestans* genes induced during a compatible infection could tell us more about the interaction at the molecular level and may include pathogenicity or virulence factors. The information we acquire about this interaction could be valuable in the develpment of protection strategies against late blight disease.

237 **Avirulence in *Venturia inaequalis*** Win, J.[2], Stehmann, C.[1], and Plummer, K.[2]. [1]HortResearch, Mt Albert Research Center, Auckland [2]Department of Biological Sciences, University of Auckland, Auckland, New Zealand

Venturia inaequalis is a fungus that causes scab disease in apple (*Malus*). We are investigating the gene-for-gene interaction between the resistance gene *Vm* originally from *Malus micromalus* and the corresponding avirulence gene *avrVm* from *V. inaequalis*. Host differential reactions are readily distinguished by a hypersensitive response (HR) in host differential h5 and large sporulating lesions (susceptibility) in host differential h1. It has been thought that the recognition event between VM and AvrVM leads to HR and hence the resistance. Our objective is to isolate and characterize *avrVm* using a reverse genetics approach. HR is induced when the cell free culture supernatant (CFCS) of *V. inaequalis* is infiltrated into leaves of h5 but not on the susceptible host h1. The HR-inducing activity is greatly reduced by proteinase K digestion but resistant to boiling. Ultrafiltration of the CFCS shows most activity is present in a fraction between 3 kDa and 30 kDa molecular weights and is contained in the supernatant after precipitation with 60% acetone. The activity has also been localized to a fraction eluting from EconoPac-Q ion-exchange column. Isoelectric focussing (IEF) gel electrophoresis shows that there are three predominant protein species present in this fraction and they have very low p*I* (approximately between 3.0 and 3.4). The N-terminal amino acid sequences have been obtained from two of the three proteins. Both show identical sequences. A degenerate primer has been designed to isolate the cDNA encoding for the protein(s) using a PCR-based strategy. A genomic library will be screened with this cDNA to isolate a full length putative *avrVm* gene. The identity of the putative *avrVm* will be confirmed by complementation experiments.

238 **The avirulence gene *ACE1* (*AVR1-IRAT7*) of the rice blast fungus *Magnaporthe grisea* encodes a polyketide synthase.** Heidi U. Böhnert, Isabelle Fudal, Waly Dioh, Didier Tharreau*, Jean- Loup Notteghem* and Marc-Henri Lebrun Physiologie Cellulaire Végétale, UMR 1932 CNRS/Biotechnologies - Aventis CropScience, 14 rue Pierre Baizet, 69009 Lyon, France. *UR-PHYMA, CIRAD-CA, Montpellier.

Resistance of rice to the rice blast fungus *Magnaporthe grisea* is governed by specific interactions between fungal avirulence genes and corresponding plant resistance genes. Genetic studies using the rice pathogenic *M. grisea* isolates Guy11 and ML25 which were fertile in crosses led to the identification of the avirulence gene *AVR1-IRAT7* (1) that interacts with the rice resistance gene *Pi11-t*. *AVR1-IRAT7* maps to chromosome one at 30cM from *AVR1 CO39* (2). The gene was isolated by positional cloning and complementation analysis. A genomic fragment of 15.5 kb able to confer avirulence to virulent strains was found to contain only one large ORF termed *ACE1*. Disruption of *ACE1* renders the avirulent parent Guy11 virulent. In the virulent parent, insertion of a retroposon has occurred at the *ACE1* locus. Sequence analysis revealed that *ACE1* encodes a large multifunctional enzyme possessing several domains characteristic of polyketide synthases. Expression of *ACE1* is specific for the penetration of leaves and artificial membranes. We propose that it is not the *ACE1* gene product itself, but a secondary metabolite which serves as the signal which is recognized by resistant host plants. *ACE1* therefore represents a new class of fungal avirulence genes. 1. Silue D. et al. 1992. Phytopathology 82: 1462-1467. 2. Dioh W. et al. 1999. Molecular Plant-Microbe Interactions. 13: 317-327.

239 **Vitamin Deficient Mutants of the Fungal Wheat-Pathogen, *Mycosphaerella graminicola.*** Joanne Ayriss[1], John Hargreaves[1] and Andy Corran[2]. [1]IACR-Long Ashton Research Station [2]syngenta, Jealott's Hill Research Station, Bracknell, Berkshire, RG42 6EY

The fungal pathogen *Mycosphaerella graminicola* (Fuckel) J. Schröt. in Cohn, (anamorph: *Septoria tritici* Rob. Ex Desm) causes leaf blotch in Wheat. It is an important economic disease causing significant yield loss throughout the World. The pathogen is mainly controlled by the application of protectant and eradicant fungicides, therefore it is important to develop new fungicides with novel modes of action to help manage and reduce the risk of a build up of resistant strains.
Enzymes of the thiamine biosynthetic pathway could provide new potential fungicide targets to be developed against crop diseases such as *M. graminicola*. The aim of this project is to validate thiamine biosynthesis as a potential target for fungicide intervention by determining whether the genes of the thiamine biosynthetic pathway are important pathogenicity factors in *M. graminicola*.

240 **Expression of the cercosporin resistance gene crg1 as a dicistronic unit with a functionally unrelated gene in the phytopathogenic fungus *Cercospora nicotianea*.** Kuang-Ren Chung[1, 2], Marilyn Ehrenshaft[1] and Margaret E. Daub[1]. [1]Department of Plant Pathology, North Carolina State University, Raleigh, NC [2]Current address: Citrus Research and Education Center, IFAS, University of Florida, Lake Alfred, FL

The *C. nicotianae* crg1 gene was identified as a gene required by the fungus for resistance to a perylenequinone toxin, cercosporin. Northern analysis identified transcripts of two sizes (4.5 and 2.6 kb) using crg1 probes, suggesting that crg1 was co-transcribed with an upstream or downstream gene. The 2.6 kb transcript (expected size for crg1) was present in very low amounts. Sequence analysis identified two ORFs flanking crg1. Using the upstream gene as a probe, we identified transcripts of 2.2 and 1.8 kb. However, two transcripts of 4.5 and 2.0 kb were identified when the downstream gene was used as a probe, suggesting that the 4.5-kb transcript is likely a co-transcribed mRNA. The downstream gene displays strong amino acid homology to a uracil transporter in yeast, and is therefore designated put1 (putative uracil transporter). The 4.5-kb transcript could be identified using either a crg1 or put1 gene probe, and was constitutively expressed in different media. The 2.6- kb transcript, also expressed constitutively, was identified only by the crg1 probe. The 2.0-kb transcript was identified only by the put1 probe and was not expressed in complete medium or V8 medium. The cDNA fragments composed of crg1 and put1 were amplified by RT-PCR directly from the 4.5-kb mRNA using one primer from the crg1 sequence and a second primer from the put1 sequence. Four cDNA clones derived from RT-PCR were sequenced, and revealed that a 64-bp fragment was spliced out in the junction of crg1 and put1. Northern analysis of three crg1-disruption mutants using the crg1 probe indicated that both the 4.5 and 2.6-kb transcripts were missing. Using the put1 probe, the 2.0-kb transcript, but not the 4.5-kb transcript, was detected in the three crg1- disrupted mutants. Taken together, we conclude that the 4.5-kb transcript is a dicistronic mRNA of both crg1 and put1. To our knowledge, this is the first reported case of a dicistronic transcript identified from filamentous fungi. Both genes can also be expressed independently as individual genes and are regulated differently. Whether or not the 4.5-kb message is translated into a fusion protein has not yet been determined.

241 **Identification of pathogenicity genes in *Heterobasidion annosum* using expressed sequence tags (ESTs).** Magnus Karlsson[1], Åke Olson[1] and Jan Stenlid[1]. [1]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden

The basidiomycete fungus *Heterobasidion annosum* is the causal agent of annosum root rot and is economically the most important disease of coniferous forests in northern temperate regions. *H. annosum* is a wood-decaying pathogen which can utilize a variety of carbon sources, such as starch, cellulose and other glucans, pectin, lignin and various phenolic compounds. It is known to secrete a wide range of extracellular enzymes and toxins. The purpose of this research project is to investigate the pathogenicity of *H. annosum* and to identify key factors that enables the fungus to infect and cause disease. A cDNA-library was constructed from *H. annosum* mycelia, where pathogenicity genes were induced by *Pinus sylvestris* seedling roots. Partial sequencing of individual cDNA-fragments yields expressed sequence tags (ESTs) and these tags are then used in homology searches against available sequence information in public databases. This will give an idea of the genes expressed during the infection process. In order to identify genes that are important for the pathogenicity of the fungus the cDNA-fragments will be screened for differential expression, comparing induced and control mycelia. A further study of putative pathogenicity genes will

include extensive expression profiling. The results from the first sequencing indicate that the cDNA-library is of good quality and we plan to sequence at least 1000 cDNA clones. In the first 200 clones fragment lengths are spanning from 350 bp to 2500 bp, with an average of 900 bp. So far 35 % of the sequences does not show any significant homology to characterized genes, while 34 % seem to be involved in protein synthesis and 31 % show significant homology to other genes.

242 **cAMP signaling and sporulation in *Ustilago maydis.*** Katherine Wake and Jim Kronstad. Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., V6T 1Z3, Canada.

The basidiomycete *U. maydis* switches from a non-pathogenic haploid yeast- like phase to a pathogenic filamentous dikaryon as a result of mating between two compatible haploid cells. Dikaryotic cells are able to proliferate in the host plant, initiate tumor formation and undergo another morphological change that results in the formation of melanized, diploid teliospores.

In general, it has not been possible to obtain teliospore formation of *U. maydis* outside the host plant and the signals that control sporulation are not well characterized. Kusch and Schauz (Crypt. Bot 1989: 1: 230-235) reported that *U. maydis* is also able to produce a haploid spore upon growth in medium with a reduced level of nitrogen. These spores have been called chlamydospores and they appear similar to teliospores in that they are rounded and melanized. However, unlike the other morphological stages of the *U. maydis* life cycle, chlamydospores are poorly understood and it is not known whether the mating loci or the cAMP /PKA pathway are involved in this morphological switch. We have found that a process which resembles chlamydospore formation in *U. maydis* can be triggered by the addition of the solvent dimethyl formamide (DMF) to cultures. Using DMF, cells with mutations in components of the cAMP pathway were examined for their ability to develop chlamydospore like cells. Cells defective in *ubc1* (the regulatory subunit of PKA) were unable to develop chlamydospores in response to DMF. Cells with defects in both *ubc1* and *adr1* (a catalytic subunit of PKA) respond to DMF by the production of chlamydospores suggesting that *adr1* functions in a pathway which inhibits chlamydospore development. Understanding chlamydospore formation may provide a model to explore the genetics of *U. maydis* sporulation, a late but crucial stage of disease development.

243 **PLS1, a gene encoding a tetraspanin-like protein, is required for penetration of rice leaf by the fungal pathogen *Magnaporthe grisea***. Mathieu Gourgues, Joaquim Cots, Pierre-Henri Clergeot, Marie-Pascale Latorse, Francoise Laurans , Régis Pépin* and Marc-Henri Lebrun UMR 1932 CNRS-Aventis and Fungicides Dpt, Aventis CropScience, Lyon, France. *Universit Claude Bernard, Lyon, France.

Unravelling functions implicated in the infection process of plant pathogenic fungi is an important challenge for crop protection. We are using as a model the fungal plant pathogen Magnaporthe grisea responsible for a major disease of rice. In order to identify functions required for the infection process, we have studied non-pathogenic mutants obtained by plasmid-mediated insertional mutagenesis. Among 3000 REMI transformants, we recovered 32 mutants either non-pathogenic (n: 10), or significantly reduced in their pathogenicity (n: 22). Only 30% of these mutants were tagged by the plasmid. We studied one of these mutants called punchless. This mutant formed melanized appressoria that failed to breach either leaf epidermis or artificial membranes such as cellophane. punchless appressoria did not differentiate penetration pegs, but had a cellular ultrastructure, turgor and glycogen content similar to wild type appressoria before penetration. punchless also failed to invade wounded or infiltrated leaves. The inactivated gene, PLS1, encodes a putative integral membrane protein of 225 amino acids (Pls1p) related to the tetraspanin superfamily. These animal proteins are components of membrane signaling complexes involving integrins that control cell differentiation, motility and adhesion. Therefore, this type of signaling pathway could be involved in penetration peg formation. The expression pattern and the intracellular localization of Pls1p were assessed to understand the role of tetraspanins in appressorium function. Pls1p was only detected in appressorium during the penetration process. Localization of Pls1p and biochemical characterization of this protein will be presented.

244 **WdChs2p, a class I chitin synthase, together with WdChs3p (Class III), contributes to virulence in *Wangiella* (*Exophiala*) *dermatitidis*, a model black pathogen of humans**. Z. Wang[1], L. Zheng[1], H. Liu[1], M. Hauser[2], S. Kauffman[2], J. M. Becker[2], and P. J. Szaniszlo[1]. The University of Texas at Austin, TX[1], The University of Tennessee, Knoxville, TN[2].

We have previously reported that *W. dermatitidis* has at least four chitin synthase genes (*WdCHS*), which we designated *WdCHS1* (class II), *WdCHS2* (class I), *WdCHS3* (class III) and *WdCHS4* (class IV). After each was cloned and characterized, they were also disrupted singly and in many combinations using various strategies. In this report, we compare the effects of disrupting *WdCHS2* and *WdCHS3* singly and together on the viability, growth, morphology, chitin synthase (WdChs) activities and virulence of this polymorphic agent of phaeohyphomycosis. As with the *wdchs2* and *wdchs3* single mutants, the *wdchs2wdchs3* double mutant cultured at 25 C or 37 C grew at wild- type rates and showed no morphological defects when grown as yeasts, hyphae or sclerotic bodies. However, assays showed that the same disruption strains were reduced in WdChs activity, with the double mutant having the least activity. These assays, together with those of a *wdchs1wdchs3wdchs4* triple disruption mutant that allowed WdChs2p to be evaluated alone, also showed that WdChs2p contributed the majority of the WdChs activity. Although no loss of virulence was detected when the single disruption strains were tested in mice, the *wdchs2wdchs3* double mutants were significantly less virulent. The importance of the WdChs2p and WdChs3p isozymes to virulence was finally confirmed by reintroducing either *WdCHS2* or *WdCHS3* into the double mutant and reconstituting wild-type virulence.

245 **Characterisation of a large polygalacturonase gene family in the oomycete plant pathogen *Phytophthora cinnamomi*.** Arvid Goetesson, Jerry S Marshall, David A Jones and Adrienne R Hardham. Plant Cell Biology, Research School of Biological Sciences, Australian National University, Canberra, Australia.

Polygalacturonases (PGs) are secreted by filamentous plant pathogens at early stages of plant infection, and their pectinolytic activity is believed to play a major role in tissue invasion and maceration. Oomycete plant pathogens are economically and ecologically important but little is known about the biochemistry and molecular biology of oomycete pathogenesis. PG activity was demonstrated in culture filtrates of the oomycete *Phytophthora cinnamomi*. A polygalacturonase gene family in *P. cinnamomi* was partially isolated and characterized. Sixteen complete *pg* genes and three incomplete sequences were isolated. Thirteen of the genes occurred in three clusters. Since *P. cinnamomi* is a diploid organism, some *pg* sequences which are > 99% identical could be alleles, but this is unlikely since the sequence context is different for all isolated *pg* sequences. DNA gel blots indicated that there may be more than 19 *pg* genes in the *P. cinnamomi* genome. Many of the predicted mature PGs had N-terminal extensions which contained repeats but were dissimilar in length and sequence. Predicted pI values varied between 4.1 and 9.3. Ten PGs had none or one potential *N*-glycosylation sites, while five had 10 to 14 potential *N*-glycosylation sites. Two *pg* genes were expressed in yeast and shown to encode functional PGs. Oomycetes are phylogenetically distinct from true fungi and more closely related to brown algae. However, phylogenetic analysis of PGs positioned *P. cinnamomi* PGs close to fungal endoPGs and distant to plant PGs. Three amino acid residues which have been subject to diversifying selection in the *P. cinnamomi* PG protein were identified.

246 **Highly conserved virulence-related *CPS1*homologs from plant and human pathogenic fungi.** Shun-Wen Lu, B. Gillian Turgeon, and Olen C. Yoder. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The *Cochliobolus heterostrophus* gene *CPS1* encodes a protein with similarity to nonribosomal peptide synthetases. We have identified *CPS1* homologs in 34 fungal species belonging to 16 genera, closely or distantly related to *Cochliobolus*. These include both plant pathogens of economic importance (e. g., *Fusarium graminearum, Magnaporthe grisea*) and human pathogens of medical importance (e. g., *Candida albicans* and *Coccidioides immitis*). Four *CPS1* homologues were cloned; three from phytopathogenic fungi, including the wheat head scab fungus *Fusarium graminearum* (*FgCPS1*, 6003 bp), the potato early blight fungus *Alternaria solani* (*AsCPS1*, 2369bp) and the barley net blotch fungus *Pyrenophora teres* (*PtCPS1*, 2306 bp). The fourth was cloned from the human pathogenic fungus *Coccidioides immitis* (*CiCPS1*, 2435 bp). Sequence analyses indicated that these genes share at least 80 % amino acid similarity and contain a highly conserved modular structure as do nonribosomal peptide synthetases. Targeted gene disruptions have confirmed that *CPS1* is required for pathogenesis of at least three different plant pathogens i.e., *C. heterostrophus* to corn, *C. victoriae* to oats and *Fusarium graminearum* to wheat. We propose that *CPS1* controls biosynthesis of a general fungal virulence factor (yet unidentified), which is required for pathogenesis by a wide array of fungi, including both plant and human pathogens.

247 **Interaction of the triazole fungicide, flutriafol, with *Leptosphaeria maculans*, the cause of blackleg disease in oilseed Brassicas.** Katherine M. Adams, Barbara Howlett, Anthony Bacic, Philip Salisbury. University of Melbourne, School of Botany, Melbourne, Australia

Leptosphaeria maculans, (anamorph *Phoma lingum*) is an ascomycete which causes blackleg disease in oilseed Brassicas. In Australia the triazole fungicide flutriafol, which acts by inhibiting 14 alpha - demethylation in ergosterol biosynthesis, is the only fungicide registered for control of this disease. The purpose of this work is to examine the biochemical interaction between the fungicide and fungus, and to elucidate genetic mechanisms which may confer triazole resistance. Seven *L. maculans* mutants with flutriafol resistance have been produced by insertional mutagenesis (REMI) or by *in vitro* conditioning on fungicide amended media. Ergosterol biosynthesis and gene expression is being compared among these mutants and wild-type *L. maculans*. Fragments of the *L. maculans* homologues of five genes implicated in triazole resistance in other fungi have been obtained. These encode regions of 14alpha- demethylase (*ERG11*), delta ^{5,6} desaturase (*ERG3*) and three ABC-transporter proteins. RT-PCR and Northern analysis are being used to study the expression of these genes in all isolates following exposure to flutriafol. Ergosterol and its sterol precursors are being separated and identified by gas chromatography ûû mass spectrometry. Analysis has revealed ergosterol is the most abundant sterol in *L. maculans*, with four ergosterol precursors present in lower abundance. Exposure of wild-type *L. maculans* to flutriafol causes a decrease in total ergosterol and an accumulation of the sterol substrate of 14 alpha-demethylase. Kate Adams is a recipient of the Nancy Millis award, provided by the Victorian State Department of Natural Resources and Environment.

248 **Visualization of AOS accumulation induced by *Botrytis cinerea* infecting tomato and bean.** Klaus B. Tenberge, Marcus Beckedorf, Britta Hoppe, and Martina Solf. Institut fur Botanik und Botanischer Garten, Westfalische Wilhelms-Universitat Munster, Schloßgarten 3, D-48149 Munster, Germany

Botrytis cinerea causes serious diseases, called grey mould, in at least 235 plant species, including important crops. The pathogen is a typical necrotroph, inducing host cell death before invasion. Active oxygen species (AOS) have been shown to be involved in infection [1]. In addition to the induced generation of AOS during the host oxidative burst, the pathogen might produce AOS itself. The aim of the presented work is to gain insight in role and source of AOS by visualizing the spatio-temporal occurrence of AOS during pathogenesis. Spore adhesion, germination and swellings of germ tube tips attached to the surface by a matrix material as well as penetration and colonization of host leaf tissues was documented on tomato and bean. Using LM, the chloronaphthol staining and the NBT technique were applied to visualize H_2O_2 and O_2-. Using TEM, the cerium chloride technique specific for H_2O_2 resulted in electron dense precipitate of cerium perhydroxide at the interface of *B. cinerea* and host cells in infected tomato and bean leaves [2]. H_2O_2 was present in the periplasmic space, in the host cell wall and at the outer surface of the host cell as well as at the outside of the fungal wall. Specific precipitate was observed inside fungal cells that appeared vital. The H_2O_2 generating system was analyzed using specific inhibitors, suggesting that fungal superoxide dismutase contributes to the production of AOS. [1] von Tiedemann A 1997. Physiol. Mol. Plant Pathol. 50, 151-166. [2] Prins TW, Tudzynski P, von Tiedemann A, Tudzynski B, ten Have A, Hansen ME, Tenberge KB, van Kan JAL 2000. In: Fungal Pathology, pp. 33-63, Kronstad JW, ed., Kluwer Academic Publishers, Dordrecht. Our research is funded by the EC in the EU-FAIR project "Oxidative attack by necrotrophic pathogens - New approaches for an innovative and non-biocidal control of plant disease" (AOS PLANT).

249 **New Restriction Fragment Length Polymorphism (RFLP) analysis markers for typing *Aspergillus fumigatus* strains.** Camile Pizeta Semighini, Steven Park[1], David S. Perlin[1], and Gustavo H. Goldman. Faculdade de Ciencias Farmaceuticas de Ribeirão Preto, Universidade de São Paulo, Brazil and [1]Public Health Research Institute, USA.

Aspergillus fumigatus is an opportunistic fungus causing several respiratory diseases, such as allergic bronchopulmonary aspergillogis, aspergilloma and invasive aspergillosis. The later is presently a major cause of death amongst immunocompromised patients, associated with a high mortality rate (85%) even when appropriate treatment is used. The incidence of aspergillosis has increased significantly over the past two decades in parallel with the number of imunocompromised patients. Although many typing approaches have been proposed, an ideal epidemiological typing technique is not available that is applicable to a wide range of *A. fumigatus* isolates. In this study, we isolated and tested Restriction Fragment Length Polymorphism (RFLP) markers for *A. fumigatus* based on PCR-products amplified by the Random Amplified Polymorphic DNA (RAPD) primer R108. Four DNA fragments, Afd, Af5, Af4, and Af4A were amplified. The fragments Afd and Af5 were 85 % and 88 % identical at the DNA level to the Afut1 retrotransposon from *A. fumigatus*. Fragment Af4A is a duplication of the fragment Af4 and both showed similarity at the amino acid level with endonucleases from other fungal retrotransposons. We have used both RAPD with the primer R108 and RFLP assays with Afut1, Afd, and Af4A as hybridization probes to determine the

genetic relatedness of clinical isolates of *A. fumigatus* isolated sequentially from four cancer patients with recurrent aspergillosis following treatment with amphotericin B. Genetic relatedness was determined by using the Coefficient of Dice. The combination of these different methods was used to demonstrate that the isolates infecting the four patients were not identical. This approach should be valuable for molecular epidemiological investigations of *Aspergillus* infections, which should facilitate the development of preventive measures for patient management. Financial support: CNPq and FAPESP, Brazil.

250 **A pharmacological and molecular approach to the study of signal transduction in the barley powdery mildew fungus.** Gemma Priddey, Ziguo Zhang, Pushpa Chaure, Alison Hall, Emma Perfect, Sarah Gurr. *Department of Plant Sciences, University of Oxford, OX1 3RB, UK.*

Blumeria graminis f.sp. *hordei* is the causal agent of barley powdery mildew disease. Infection is spread by asexual conidia, which, on contact with the leaf surface, undergo a highly regulated and complex programme of development. As an obligate biotroph *B. graminis* cannot be grown axenically and so tissue for experiments is limiting. We have employed and described a range of techniques to assess how *B. graminis* perceives, integrates and relays signals for morphogenesis up to the point of penetration. We have demonstrated that both physical properties of the leaf surface, such as hydrophobicity, and cuticle-derived chemicals, such as cutin monomers and cellulose, promote *B. graminis* differentiation. How does *B. graminis* transduce signals to drive differentiation and development? Applications of exogenous agonists and antagonists have allowed us to demonstrate a role for cAMP signalling and PKA in germling differentiation, but this work also highlights that cAMP alone is not sufficient to trigger the complete programme of differentiation.

Furthermore, we have identified several component genes of signal transduction and cell integrity pathways in *B. graminis*, including two PKC genes, two MAPK genes and two chitin synthase genes. Their expression profiles show that they are regulated differentially during conidia germination and appressorial differentiation. They putatively play important roles in host penetration and pathogenicity. We aim to ascribe functions to these genes, by using our recently-described stable DNA transformation technique and also to study the interplay between the PKA, PKC and MAPK signal transduction pathways.

251 **Molecular genetics of plant infection by the rice blast fungus *Magnaporthe grisea*.** Nicholas J. Talbot, Eckhard Thines, Andrew J. Foster, Roland W.S. Weber, Pascale V. Balhadere, Virginie Colas . School of Biological Sciences, University of Exeter,Washington Singer Laboratories, Perry Road, Exeter, EX4 4QG.

The rice blast fungus elaborates a specialized infection structure called an appressorium in order to infect rice leaves. The appressorium is a dome-shaped cell which differentiates from a germ tube, shortly after conidial germination. *M. grisea* appressoria develop turgor pressure which is translated into mechanical force to breach the plant cuticle. This allows a narrow penetration peg to enter the leaf epidermis and colonise the tissue, later forming large bulbous infection hyphae. We are investigating the process of appressorium-mediated infection in *M. grisea* and in particular the mechanism by which turgor is generated. *M. grisea* appressoria accumulate very high concentrations of glycerol which acts as an osmolyte, allowing the cell to take up water and develop hydrostatic turgor. Appressoria form in water on the leaf surface and therefore glycerol is synthesised from storage products in the spore. *M. grisea* conidia contain a number of storage compounds including glycogen, lipid and trehalose. We have used a combination of genetic, biochemical and cell biological methods to study the relative contribution of each storage product to glycerol generation (Thines et al., 2000 Plant Cell 12, 1703-1718). Trehalose degradation occurs rapidly during conidial germination. Trehalose is synthesised in *M. grisea* by trehalose-6-phosphate synthase encoded by the TPS1 gene. Dtps1 mutants are extremely reduced in pathogenicity, due to a defect in cuticle penetration. The degradation of trehalose meanwhile appears to occur due to the activity of at least two trehalases, encoded by NTH1 and TRE1. Dtre1 and Dnth1 mutants are also affected in their ability to cause disease symptoms, although to a lesser extent than in Dtps1. Genetic control of trehalose metabolism appears to be, at least in part, due to the action of cAMP-dependent protein kinase A (PKA), based on enzymatic assays. Glycogen and lipid stores are also degraded during conidial germination and can be observed accumulating in appressoria during their formation, before disappearing as turgor is generated. The movement of these reserves is controlled by the PMK1 MAP kinase pathway and degradation in the appressorium is regulated by PKA. We are currently identifying and characterising genes encoding enzymes involved in lipid and glycogen degradation in appressoria with the aim of determining the basis of turgor generation and ap

252 **Identification and characterisation of two metallothionein-encoding genes from the rice blast fungus, Magnaporthe grisea.** Sara L. Tucker and Nicholas J. Talbot. School of Biological Sciences, University of Exeter, Washington Singer Laboratories, Perry Road, Exeter, EX4 4QG

Magnaporthe grisea is the causal agent of rice blast disease. Considerable research has led to our current understanding of this pathogen however little emphasis has been placed on identifying genes specifically involved in plant tissue colonisation and growth of the fungus in planta. I this project we have adopted tow strategies to study plant tissue colonisation by *M. grisea*. The first strategy involved differential cDNA screening to isolate transcripts expressed in the wild-type strain of *M. grisea*, Guy-11 but not in a non-pathogenic MAP kinase mutant Dpmk1. Secondly, a candidate gene approach was used to identify a homologus of a gene identified in *Uromyces fabae* called PIG11. Because characterisation of PIG11 in this obligate biotrophic fungus is difficult, the presence of a homologue in the more experimentally amenable fungus *M. grisea* is significant. Using these approaches two metallothionein (MT)-encoding genes have been identified called MMT1 (the PIG11 homologue) and MMT2. Metaolothioneins are ubiquitous proteins with metal-binding propteries, although their function is somewhat elusive. Preliminary characterisation of the two MTs carried out to date will be presented, revealing their relatedness to other MTs, their patterns of expression in developmental mutants of *M. grisea* and the possible functions they may carry out during growth of the

253 **Characterization of the MPG1 hydrophobin-encoding gene from the rice blast fungus *Magnaporthe grisea.*** Michael J. Kershaw, Darren Soanes and Nicholas J. Talbot. School of Biological Sciences, University of Exeter, Washington Singer Laboratories, Perry Road, Exeter, EX4 4 QG, United Kingdom

The rice blast fungus *Magnaporthe grisea* infects its host by elaboration of a specialised infection structure known as an appressorium. This single-celled structure forms in response to the hard, hydrophobic rice leaf surface and brings about infection due to generation of hydrostatic pressure (3). *M. grisea* appressoria are melanin-pigmented cells with a thickened cell wall that allows turgor to develop within the cell due to accumulation of glycerol and the subsequent influx of water. Mechanical rupture of the plant leaf cuticle occurs and a narrow penetration peg enters the leaf epidermis, providing the route for fungal colonization of the plant leaf tissue (3).
During appressorium development the MPG1 hydrophobin gene is highly expressed and secretion of the class I hydrophobin is important for efficient elaboration of appressoria (1, 2). A targeted gene replacement of MPG1 reduces the ability of *M. grisea* to elaborate appressoria and mutants are consequently reduced in their ability to cause disease symptoms (1). Dmpg1 mutants also show reduced surface hydrophobicity and lack a conidial rodlet layer, indicating that MPG1 performs a number of roles in the developmental biology of *M. grisea*. The importance of differential expression of MPG1 to *M. grisea* can be clearly seen by the fact that numerous class I hydrophobin genes are able to functionally complement Dmpg1 mutants, restoring pathogenicity and appressorium development, but only if expressed under control of the MPG1 promoter (4). This suggests that class I hydrophobins, which are widely distributed among fungi and may perform orthologous functions (5, 6). The close functional relationship of hydrophobins is somewhat surprising considering the low level of amino acid homology among the group and emphasises the role of differential gene expression in determining hydrophobin function during fungal morphogenesis.
Regulation of MPG1 expression occurs due to the action of a number of cis-acting sequences and trans-acting factors which control MPG1 transcript levels. The latter include the NPR1 and NPR2 gene products which regulate pathogenicity of M grisea and the starvation stress response (5). The significance of MPG1 regulation and the role of specific amino acid residues in the function of the MPG1 hydrophobin will be discussed.
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254 **Characterization of pathogenicity factors of *Fusarium oxysporum* f.sp. *lycopersici.*** Roselinde G E Duyvesteijn, Yvonne W Boer, Menno van der Voort, Ben J.C. Cornelissen, Michel A Haring. University of Amsterdam, Plant Pathology, Amsterdam, Noord-Holland, The Netherlands

Fusarium oxysporum is a soilborne plant pathogenic fungus which can cause considerable economical loss on a farmers crop. *Fusarium oxysporum* forma specialis *lycopersici*(Fol) infects only tomato plants. Until now there are three different races of Fol known which are all pathogenic on tomato. During the infection *Fusarium*uses

pathogenicity genes which are necessary to penetrate, invade and colonize the tomato roots. Characterizing the pathogenicity factors could give a deeper understanding of the infection process of the fungi. In order to characterize these genes insertional mutagenesis will be performed. However, by using a promoterless construct it is possible to select planta induced genes which are the most likely pathogenicity factors. Therefore, a promoterless -glucuronidase (*GUS*) gene was cloned into a plasmid containing the hygromycin resistance selection marker. The new plasmid was transformed into the Fol race 2. In total 216 transformants were selected for their ability to grow on medium with hygromycin. Bioassays identified19 putative mutants which had reduced pathogenicity. Southern blots will be done to analyze the number of copies of the inserted plasmid and PCR experiments can be used to confirm the presence of the hygromycin and GUS genes. The inserted plasmid and its flanking DNA regions will be cloned of the non-pathogenic transformants. The involvement of the flanking DNA in pathogenesis will be established by making knock-out mutants.

255 **Signaling and pathogenicity in the gray mold *Botrytis cinerea.*** Christian Schulze Gronover, Annett Klimpel, Daniela Kasulke and Bettina Tudzynski.Westfälische Wilhelms-Universität Münster, Institut für Botanik, Schlossgarten 3, 48149 Münster, Germany

Botrytis cinerea is the causal agent of grey mould diseases of many economically important fruits, vegetables and flowers. Our main interest is the study of the genes involved in the interaction process between the fungus and its host plant. As a necrotrophic fungus, *B. cinerea* induces host cell death after penetrating the plant tissue. Like other fungal pathogens, *B. cinerea* senses the presence of plant surfaces and triggers the synthesis of several specific gene products in response. We suggest that several conserved signal transduction pathways such as cAMP signaling are required for infection. Therefore, we work on cloning and characterization of genes encoding members of signal transduction pathways: genes coding for G-alpha proteins, adenylate cyclase and protein kinases. Heterotrimeric G proteins play an important role in transducing several extracellular signals from activated transmembrane receptors of the cell surface to a variety of intracellular targets via a cascade of interacting proteins, such as cAMP cyclase and protein kinases. Recently, we cloned two different G-alpha protein-encoding genes, *bcg1* and *bcg2*. A 180 bp PCR fragment, obviousely coding for a third G-alpha protein, (*bcg3*), was also amplified. BCG1 showed a high degree of identity with CPG-1 from *Cryphonectria parasitica* and MAGB from *Magnaporthe grisea* and belongs to the Gai class (inhibitory G-alpha). BCG2 was grouped into the same family as MAGC from *M. grisea* and GPA-3 from *Ustilago maydis* which are not involved in the induction of infection structures. Interestingly, *bcg1* and *bcg2* are expressed *in planta* at a very early stage of infection (12 hours after drop infection). For both genes, *bcg1* and *bcg2*, knock out mutants were isolated. *bcg1* knock out-transformants showed a colony morphology clearly different from that of the wild-type. The colony margins were compact and sharply defined. Tomato and bean leaves inoculated with conidial suspensions from *bcg1*- and *bcg2*-mutants as well as from the wild-type caused similar primary necrosis lesions in the first hours. However, after two days no further increase of the diameter was observed for the lesions caused by the *bcg1* conidia compared with the fast developing wild-type and *bcg-2* necrosis spots. Interestingly, *bcg1*-mutants do not produce extracellular proteases anymore. Further infection studies and physiological tests are on the way.

In some other fungal pathogens it could be clearly demonstrated that cAMP plays an important role for successful plant infection. Therefore, a *B. cinerea* adenylate cyclase gene (bcac1) and two catalytic subunit-encoding genes of two putative protein kinase A (*bc-PKAC1* and *bc-PKAC2*) were cloned. For the *bcac1* gene, a gene replacement vector was constructed and knock out-transformants were isolated. They show reduced conidia formation and vegetative growth. The effect of the gene inactivation on the infection process on bean and tomato leaves is under investigation. The results will be discussed in the background of the role of signaling processes for the development of diseases caused by a broad spectrum of fungi.

256 ***Cochliobolus carbonum* mutants lacking various classes of cell-wall-degrading enzymes.** John S. Scott-Craig and Jonathan D. Walton. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 U.S.A.

The maize pathogen *Cochliobolus carbonum* secretes a large number of plant-cell-wall-degrading enzymes when grown with maize cells walls as the sole carbon source. Xylan-containing polymers constitute almost 50% of the dry weight of the maize cell wall, non-cellulosic glucans about 8% and pectin about 6%. At least five xylan-degrading, four glucan-degrading and three pectin-degrading enzymes are produced by the fungus. Genes encoding four endo-

xylanases (*XYL1,2,3,4*), one -xylosidase (*XYP1*), two exo-glucanases (*EXG1,2*), two mixed-link glucanases (*MLG1,2*), an endo-polygalacturonase (*PGN1*), an exo-polygalacturonase (*PGX1*) and a pectin methylesterase (*PME1*) have been cloned and used to create strains containing deletions of each gene. These deletions, singly and in combination, are being examined for their effects on the growth of the fungus *in vitro* on xylan, -glucan or pectin and on the ability of the fungus to infect maize.

257 ***Agrobacterium* T-DNA tagging of pathogenicity genes in *Fusarium circinatum*.** Sarah F. Covert, Mei-Ho Lee, and William S. Bowers. Warnell School of Forest Resources, University of Georgia, Athens, GA, USA.

Fusarium circinatum (teleomorph: *Gibberella circinata*) causes pitch canker disease on many species of pine. In particular, the native stands of Monterey pine (*Pinus radiata*) in California are severely affected by this disease. We are using *Agrobacterium tumefaciens* T-DNA gene tagging to identify the genes that make *F. circinatum* pathogenic on Monterey pine. *A. tumefaciens* strain AGL1(pPK2) transforms *F. circinatum* with an efficiency of 2 - 150 transformants/10^5 conidia. The T-DNA is integrated into the genome and is stable through mitotic and meiotic cell divisions. Seventy-five percent of the transformants contain a single T-DNA copy. The remaining 25% contain two copies of the T-DNA. We are currently screening hundreds of transformants for their ability to cause necrotic lesions on Monterey pine. If mutants lacking pathogenicity contain more than one T-DNA tag, we will use genetic segregation to isolate the tag causing the mutation of interest. Inverse PCR will be used to recover the *F. circinatum* DNA flanking the T-DNA. This approach should allow us to identify *F. circinatum* genes contributing to pitch canker symptom development.

258 ***In planta* expressed genes in the interaction between *Gaeumannomyces graminis* and cereals.** Morgane Guilleroux and Anne Osbourn., Sainsbury Laboratory

Suppression subtractive hybridization (SSH) has been used to generate a cDNA library enriched for sequences that are differentially expressed during infection of wheat roots by *Gaeumannomyces graminis*. This library has been assessed to confirm that representative constitutively expressed plant and fungal sequences (alpha-tubulin and actin, respectively) have been subtracted and XYL1 (a xylanase that is known to be expressed during infection)of *GgA* is expressed. A pilot study of the subtracted library has been carried out on 215 clones. These clones have been sequenced to check the quality of the library and subjected to a BLASTX search. Of these 215 clones, 150 reliable DNA sequences were obtained, 7 of which showed significant homology with fungal gene sequences available in the databases. However, the small average insert size (200bp) impairs both reliable homology search and hybridizations in Southern and northern blot experiments. A cDNA library has therefore been constructed from mRNA from infected roots, and is being used to isolate full-length cDNAs corresponding to subtracted clones of interest. Larger cDNAs fragments of two of the SSH clones have been used as probes on Northern blots and shown to be upregulated during infection. These clones are both of plant origin. An arrayed genomic DNA library of *Gaeumannomyces graminis* has been constructed and is being screened with the SSH library and other complex probes to gain a better understanding of the metabolic requirements of this root pathogen during the infection process. Gene function will be tested by gene disruption in related fungus *Magnaporthe*.

The Sainsbury laboratory is funded by the Gatsby Charitable Foundation

259 **Active oxygen species in the *Claviceps purpurea*/rye interaction** Sabine Moore, Suchitra Joshi, Birgitt Oeser and Paul Tudzynski. Institut fuer Botanik, Westf. Wilhelms-Universitaet, Schlossgarten 3, Muenster D-4814, Germany.

The ascomycete *Claviceps purpurea* is a biotrophic cereal pathogen which colonises young ovaries, drawing nutrients from the vascular tissue located at the ovary base, and forming a stable host-pathogen interface there. Both normal ontogenesis as well as the defence response involve production of active oxygen species (AOS) by the rye ovary; our research focusses on the ability of the fungus to overcome oxidative stress during colonisation and the importance of these mechanisms for pathogenicity. IEF gel analysis of axenic and pathogenic cultures show that the fungus contains at least three distinct catalases (1) and one Cu,Zn superoxide dismutase. Single mutants deficient in the major secreted catalase CATC/D (2) or the copper-induced, cell wall associated Cu,Zn SOD (SODA) show no significant reduction in pathogenicity; a double mutant lacking both enzymes shows in planta only a slight delay in

conidiospore production. A second catalase gene has been cloned and deleted, analysis of the mutant will show if this gene corresponds to the second secreted catalase. We are searching for further AOS induced genes using copper which acts as a fenton catalyst and thus a stable source of AOS. Using differential screening with and without copper, we have isolated several interesting genes, most of which are also induced by hydrogen peroxide. In order to better understand the mechanisms of oxidative gene induction, we have cloned a gene which encodes a transcription factor induced by hydrogen peroxide; functional analysis of this gene is underway. 1. Garre V, Tenberge KB, Eising R: Phytopathol. 88. 744-753 (1998) 2. Garre V, M ller U, Tudzynski P: MPMI. 11, 772-783 (1998)

260 **A histone deacetylase gene related to yeast *HOS2* is necessary for extracellular depolymerase expression and virulence in the fungus *Cochliobolus carbonum*.** Dipnath Baidyaroy[a], Gerald Brosch[b], J-H Ahn[a], Sigrun Wegener[a], Stefan Graessle[b], Oscar Caballero[a], Peter Loidl[b], and Jonathan Walton[a]. [a]Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, and [b]Department of Microbiology, University of Innsbruck, Medical School, A-6020 Innsbruck, Austria

The gene *ccHOS2*, encoding a putative histone deacetylase (HDAC) related to yeast *HOS2*, was isolated from the filamentous fungus *Cochliobolus carbonum*, a pathogen of maize that makes the HDAC inhibitor HC-toxin. Conidia of engineered *cchos2* mutants were smaller and less septate. HDAC activity in the mutant was decreased by ~50%. Growth of the *cchos2* mutant *in vitro* was normal on glucose and sucrose but was reduced on arabinose, xylose, xylan, pectin, and maize cell walls. The mutant produced less extracellular depolymerase activity and induction of the corresponding genes was also reduced. The *cchos2* mutant also produced fewer lesions than the wild type on susceptible maize due to reduced penetration efficiency. Except for altered conidial morphology, these phenotypes were similar to those of a *C. carbonum* strain mutated in the *ccSNF1* gene encoding a glucose-regulated protein kinase. The results show that (1) an HDAC gene can have multiple functions in a filamentous fungus, (2) HDAC genes can be required for gene induction as well as repression, (3) some HDAC genes can have specific functions, and (4) *ccHOS2* is required for virulence of *C. carbonum* on maize.

261 **Genetic and physical mapping of the Avr1a avirulence gene in *Phytophthora sojae*.** Terry MacGregor[1], Madan Bhattacharyya[2], Brett Tyler[3] and Mark Gijzen[1]. [1]Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, London, ON, Canada. [2]G303 Agronomy Hall, Iowa State University Ames, Iowa, USA, [3]Department of Plant Pathology, The University of California, Davis, CA

Compatibility between soybean and the oomycete phytopathogen *Phytophthora sojae* is controlled by host resistance (R) genes and pathogen avirulence (Avr) genes. The segregation of the Avr1a avirulence gene in *P. sojae* F2 populations is consistent with that of a dominant allele at a single locus. By using random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and bulked segregant analysis, the Avr1a locus was mapped in two F2 populations derived from four different parental isolates of *P. sojae*. Four RAPD and nine AFLP markers linked to Avr1a were identified from an estimated 2100 RAPD and 40,000 AFLP loci screened. Segregation analysis of all 13 markers in one F2 population of 90 progeny generated a map of 113.2 cM encompassing Avr1a, with the closest marker co-segregating with the trait. Chromosome walking was initiated using the co-segregating marker as a probe to screen a *P. sojae* bacterial artificial chromosome (BAC) library, and a contig of eight clones, spanning 170 kb, was constructed. Three markers derived from the contig, covering a distance of 120 kb, co-segregated with Avr1a in an enlarged F2 population of 199 progeny. Thus, the ratio of physical distance to genetic distance appears greater than 120 kb/cM in this region of the *P. sojae* genome. Analysis of *P. sojae* genomic DNA blots, using the markers as hybridization probes, revealed that all of the markers tested represent single or low copy sequences and showed a high level of conservation around the Avr1a locus in all four races studied, plus nine other races. This study has laid the foundation for a map-based cloning strategy for isolating Avr1a.

262 **The pisatin demethylases from *Nectria haematococa* MPVI and *Fusarium oxysporum* f.sp. *pisi* are highly similar**. Catherine C. Wasmann[1], Kevin McCluskey[2] and Hans D. VanEtten[1]. [1]Department of Plant Pathology, Room 204 Forbes Building, University of Arizona, Tucson, Arizona 85721 U.S.A. [2]Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City, Kansas 66160.

Several fungal pathogens of pea are able to degrade the pea phytoalexin pisatin thus detoxifying it. This detoxification is best characterized in the fungus Nectria haematococca where it has been shown that isolates

pathogenic on pea can detoxify pisatin via a cytochrome P450-mediated demethylation. Seven genes (PDA) for pisatin demethylase have been identified in *N. haematococca* by classical genetics and three of these genes have been sequenced. Whereas the gene (PDA) for pisatin demethylase from *N. haematococca* is highly specific for pisatin as an inducer and repressed by glucose, the PDA genes from other fungal pathogens are, in general, neither specific for pisatin nor strongly repressed by glucose. A PDA gene from another fungal pathogen of pea, *Fusarium oxysporum* f.sp. *pisi* isolate T415, has been sequenced and its deduced amino acid sequence found to be 89% similar to that of the PDA1 gene of *N. haematococca*. The ability of pisatin and several related compounds to induce the gene from *F. oxysporum* has been tested in vivo and, like the PDA1 gene from *N. haematococca,* the gene from *F. oxysporum* appears to be specific for pisatin. However, in contrast to the PDA1 gene from *N. haematococca,* induction of the *F. oxysporum* gene by pisatin is not repressed by glucose and, in this respect, the PDA gene from T415 appears similar to the pisatin demethylases of other pea pathogens.

263 **MAP kinases in signal transduction pathways: cloning and functional analyses of PMK1 and MPS1 homologues from *Claviceps purpurea*.** G. Vautard-Mey[1], B. Oeser[1], M. H. Lebrun[2] and P. Tudzynski[1]. [1] Westfaelische Wilhelms-Universitaet Muenster, Institut fuer Botanik, Schlossgarten 3, 48149 Muenster, Germany. [2] UMR 1932 CNRS-Aventis, Physiologie Cellulaire V g tale, 14 rue P. Baizet, 69009 Lyon, France.

The early infection process of phytopathogenic fungi may involve both cell wall components able to recognise the host surface as well as signal chain elements triggering differentiation of infection structures and subsequent tissue colonisation. MAP kinases play a central role in sensing and transducing extracellular signals important for the regulation of gene expression. *Claviceps purpurea*, a common biotrophic pathogen of cereals responsible for the ergot disease, is used as a model to identify components of pathogenicity-related signalling pathways. Degenerate primers were used to amplify two genomic fragments whose deduced peptide sequences are homologous respectively to Pmk1 and Mps1, two MAP kinases essential for the pathogenicity of *M. grisea*. Both PCR probes were used to screen a *C. purpurea* genomic library. *Cpmk1* and *Cpmk2* (*Claviceps purpurea* MAP kinase genes 1 and 2) were subsequently isolated and sequenced. Their deduced peptide sequences show 95 % and 84 % identity to Pmk1 and Mps1, respectively. In order to determine the importance of both kinases for the pathogenicity of *C. purpurea*, two replacement vectors were constructed and used to transform the wild type strain. Knock-out mutants were obtained for both genes. Determination of their pathogenicity is now in progress. The expression of *Cpmk1* and *Cpmk2* during infection of rye is also being studied using RT-PCR. In order to determine whether *Cpmk1* is able to restore appressoria formation in the *M. grisea* delta*pmk1* mutant strain, complementation experiments are being performed. Similar experiments will be carried out using *Cpmk2* and the *M. grisea* delta*mps1* mutant which is affected in secondary hyphae formation. As *C. purpurea* doesnÆÆt form special infection structures, such experiments could provide insight on the functional homology of kinases conserved in pathogenic fungi which have evolved different modes of infection.

264 ***Colletotrichum graminicola* pathogenicity mutants identified using restriction enzyme mediated integration (REMI).** Michael R Thon, Etta Nuckles and Lisa J. Vaillancourt. University of Kentucky, Department of Plant Pathology, Lexington KY, USA. 40546-0091.

A collection of approximately 1600 REMI transformants of the plant-pathogenic fungus *Colletotrichum graminicola* was screened for mutants that were reduced in pathogenicity to maize stems and leaves. Three non-pathogenic mutants have been identified as well as six mutants with reduced pathogenicity. Many transformants were reduced in growth rate and spore germination rate however these factors were not correlated with pathogenicity. The mutants contain defects in both pre- and post-infection stages of pathogenesis, including spore germination, appressorium formation, and host tissue colonization. One non- pathogenic mutant is able to complete host infection but is unable to colonize host tissue. Genomic DNA flanking the plasmid integration site of this mutant was used to identify corresponding clones in a wild-type genomic library. Pathogenicity of this mutant was restored when it was transformed with a 6 kb subcloned DNA fragment. Sequence analysis indicates that this strain contains a mutation in a putative homologue of the yeast SPC3 gene, which encodes a component of the yeast signal peptidase, involved in processing of protein secretion signals.

265 **Structural and functional analysis of an oligomeric hydrophobin gene in *Claviceps purpurea*.** G. Vautard-Mey[1], B. Oeser[1], T. Correia[1], M. J. Kershaw[2], N. J. Talbot[2] and P. Tudzynski[1]. [1] Westfaelische Wilhelms-

Universitaet Muenster, Institut fuer Botanik, Schlossgarten 3, 48149 Muenster, Germany. [2] School of Biological Sciences, University of Exeter, Washington Singer Laboratories, Exeter EX4 4QG, United Kingdom.

Hydrophobins are small secreted proteins ubiquitous to filamentous fungi. Fungal aerial structures such as aerial hyphae, conidia and fruiting bodies are coated with an amphipathic layer, resulting from the self-assembly of hydrophobins on the outer cell wall. Among the many functions they may have, hydrophobins were shown to be implicated in aerial hyphae emergence, adhesion to hydrophobic surfaces and interaction of phytopathogens with their host. An unusual gene, encoding a modular protein consisting of five hydrophobin units separated by GN-repeats, was identified in *C. purpurea*, a biotrophic fungus responsible for the ergot disease of cereals. Until now, only the *C. fusiformis* trihydrophobin shows a comparable structure (**1**). The three internal units share more than 90 % identity at the nucleotidic level, suggesting that the protein might result from recent duplication events which occurred in a tripartite precursor. Sequencing of cDNA clones revealed that the main pentahydrophobin transcript codes for the full- length protein. Hydrophobins can be divided into two classes of different biochemical properties, according to the spacing of eight conserved cysteine residues. Due to its modular structure, the *C. purpurea* pentahydrophobin, composed of five class II units, could have unusual biochemical properties. Complementation experiments are being performed, using the *Magnaporthe grisea* delta*mpg1* mutant strain lacking a monomeric class I hydrophobin. Mgp1 is until now the sole hydrophobin which has been shown to be required for pathogenicity. The pentahydrophobin gene (*Cpph*) was shown to be expressed during infection of rye (**2**). In order to determine its importance for the pathogenicity of *C. purpurea*, a replacement vector was constructed and used to transform the wild type strain. Mutants lacking *cpph* were isolated and analysis of their pathogenicity is in progress.

(**1**) de Vries, O. M. H., Moore, S., Arntz, C., Wessels, J. G. H. and Tudzynki, P. (1999) Eur. J. Biochem. 262: 377-385. (**2**) Tenberge, K. B., Stellamanns, P., Plenz, G. and Robenek, H. (1998) Eur. J. Cell Biol. 75: 265-272.

266 **Cloning and characterization of two subtilisin-like protease genes from the fungal endophyte *Neotyphodium lolii.*** Michelle McGill and Barry Scott. Institute of Molecular BioSciences, Massey University, Private Bag 11 222, Palmerston North, New Zealand

Subtilisin-like proteases play important roles in fungal nutrition and pathogenicity. Although the role of these proteases is well established in fungal interactions with insects, nematodes and other fungi, little is known about their role in plant-fungus interactions. The plant-fungal interactions where these proteases have been detected include the endophyte *Epichlo typhina*and its host grass *Poa ampla,*and the grass pathogen *Magnaporthe grisea* and its host, *Poa pratensis.*In both cases the fungus produces high levels of subtilisin-like proteases suggesting that these enzyes have an important biological role. We have cloned two subtilisin-like protease genes from *N. lolii,*a fungal endophyte of perennial ryegrass. The *prt1*gene encodes a protein with 49% identity to proteases from *Aspergillus*species. Downstream of *prt1*is a 360 base pair minisatellite that consists of forty copies of a 9 base pair motif. The *prt2*gene encodes a protein with 56% identity to proteases from entomopathogenic fungi. The subtilisin-like proteases in this group act as virulence factors by breaking down the physical barriers to fungal infection and also provide a source of nutrition. RT-PCR has been used to analyze expression of *prt1*under a range of nutritional conditions. Results show that under conditions of high nutrient availability *prt*genes may not be expressed, while when preferred carbon or nitrogen sources are lacking expression of the *prt*genes is derepressed.

267 **Cloning a protein kinase from *Cryphonectria parasitica* and its transcriptional regulation by hypovirus.** Jin-Won Choi, Myoung-Ju Kim, Seung-Moon Park, Moon-Sik Yang, and Dae-Hyuk Kim. Chonbuk National University, Biological Sciences, Chonju, Chonbuk, Korea

The gene encoding Ser/Thr protein kinase (cpk-1) was isolated from chestnut blight fungus, *Cryphonectria parasitica.* Degenerated primers for cppk-1 was designed based on conserved catalytic domain of many fungal PK. The expected size of 350-bp amplicon was obtained by using PCR and cloned into plasmid vector. Sequence comparison of the cloned fragment showed the highest similarity to ptk1, Ser/Thr protein kinase from Trichoderma reesei, with 82% identity. The cpk-1 consisted of three exons with two intervening sequences of 67 bp and 71 bp in size, and the deduced cppk-1 protein product, CPK-1, had an estimated molecular mass of 70.5 kDa and a pI of 7.45. Primer extension experiment revealed the major transcription initiation site located at -49 bp from the translation start codon and the sequencing of cDNA clone indicated the poly(A) occurred at 569 bp down-stream of the stop codon. Northern blot analysis and RT-PCR were conducted to examine the expression pattern of cpk-1 from virus-

free *C. parasitica* strain EP155/2 as well as its isogenic hypovirulent strain UEP1. The cpk-1 expressed at low level at both 1 and 5 day after the liquid culture while the cpk-1 expression increased considerably at 5 day by the presence of the hypovirus CHV1-713. This indicates the implication of hypovirus in the signal transduction pathway of *C. parasitica* through a modulation of cppk-1 expression and it is one of the first evidence showing that the hypovirus disturbs fungal signal transduction pathway at the transcriptional level. Correlation of an aberrant expression of cpk-1 to the specific viral symptoms is under investigation. M-J Kim was supported by Brain Korea 21 program from the Korean Ministry of Education.

268 **Use of *Ac/Ds*based gene traps for identification and characterization of pathogenicity factors from the rice-blast fungus, *Magnaporthe grisea*** Naweed I. Naqvi.,[1] Shanthi, S.,[1] Weil, C.,[2] Kunze, R.,[3] Sundaresan V [1]. [1]Institute of Molecular Agrobiology, 1 Research Link, National University of Singapore, Singapore 117604, [2]Dept. of Biological Sciences, University of Idaho, Moscow, ID83844, USA; [3]Institut fur Genetik und Mikrobiologie, Universitat Munchen, Maria-Ward-Strasse 1a, 80638 Munich, Germany

In *Magnaporthe grisea*, the causal ascomycete of blast disease on rice, the pathogen cycle involves the following developmental sequence: deposition of a conidium on the rice leaf, germination of the conidium to form a germ tube, differentiation of the germ tube into a specialized infection structure called appressorium, penetration of the leaf surface by the melanized appressorium via a penetration peg, differentiation of the penetration peg into secondary hyphae leading to subsequent colonization of plant tissue. In this developmental sequence, only the melanization and pressurization of the appressorium is well understood (Annu. Rev. Microbiol., 50:491-512, 1996). The molecular basis of the infection-related development of a plant pathogenic fungus has not been fully explored, and elucidation of the underlying basis of this stimulus-response relationship at the structural and regulatory level would help us gain insights into mechanisms controlling the infection process and would lead to development of novel disease control strategies. We are using a novel insertional mutagenesis approach to explore the molecular basis of infection-related development in *M. grisea*. Towards this end, we have developed a system in which transposition of the maize*Ac/ Ds*elements occurs in *M. grisea*. Evidence for successful *Ac/Ds* transpositions in the blast fungus shall be presented alongwith strategies for their use as valuable insertional-mutagenesis tools in identifying genes involved in pathogenicity of the rice-blast fungus. Preliminary data on the use of Agrobacterium T-DNA mediated random insertions in *Magnaporthe* shall also be discussed.

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269 **Virulence factors and defense proteins in tomato xylem sap during colonization by *Fusarium oxysporum*.** Martijn Rep, Petra Houterman and Ben J. Cornelissen. University of Amsterdam, Plant Pathology, Amsterdam, The Netherlands.

Fusarium oxysporum colonizes tomato plants through invasion of xylem vessels. Whether the fungus will colonize the entire plant, resulting in wilt disease, or is restricted to one or only a few vessels, is determined by recognition events taking place within vessels . Proteins secreted by the fungus in xylem sap are likely to play a crucial role here. On the one hand, they can serve to promote fungal colonization, for instance through degradation of plant cell walls or suppression of plant defense mechanisms. On the other hand, some of these proteins may in fact induce plant defense reponses when they are recognized by plant cells. Such elicitors of plant defense responses have not yet been identified for *Fusarium* or any other fungal vascular pathogen.

In order to better understand the molecular basis of *Fusarium* pathogenicity, we aim to identify the full spectrum of proteins excreted by the fungus in tomato xylem vessels. In addition, we wish to identify plant proteins that are produced during disease development, in order to have a view on the strategies employed by the plant to suppress colonization. We have recently undertaken steps towards this goal. Xylem sap protein patterns from healthy and *Fusarium*-infected plants were compared by SDS-PAGE, revealing candidates for race-specific *Fusarium* proteins as well as plant-produced 'defense' proteins. Identification of these proteins with Mass Spectrometry is in progress.

270 **Gene expression during pre-symbiotic development of arbuscular mycorrhizal fungi** M'Barek Tamasloukht, Guillaume Becard* and Franken Philipp. Max-Planck-Institut fur terrestrische Microbiologie, Karl-

von-Frisch-Strasse, 35043 Marburg, Germany *Equipe de Mycologie Vegetale, UMR5546 CNRS/Universite Paul Sabatier, Pole de Biotechnologie Vegetale; 24, chemin de Borde-Rouge BP Auzeville, France

Arbuscular mycorrhizas (AM) are symbiotic associations formed between vascular land plants and fungi of the order Glomales (Zygomycota). These associations can be found on the majority of vascular plant species and represent therefore key elements of most terrestrial ecosystems. Unfortunately, AM fungi cannot be propagated in pure culture, since they are obligate symbionts and need to colonise roots to fullfill their life cycle. Nevertheless, they germinate without the host and show limited growth of hyphae during presymbiosis. This growth arrests after approximately two weeks, but hyphal elongation and branching can be induced by addition of root exudates. In order to investigate the molecular basis of presymbiotic hyphal development, we are analysing gene expression in the AM fungi *Gigaspora rosea* and *Gig. Gigantea* by two methods, differential RNA display and suppressive substractive hybridisation. Up to now, more than 600 clones were obtained which contain cDNA fragments with an average size between 150 and 1500 bp. Randomly selected clones were screened by reverse Northern blot, in order to identify genes upregulated by the addition of root exudates. Differential expression of selected genes mainly involved in signal transduction processes and in respiration has been verified by RT-PCR. In parallel, cytological investigations are carried out to prove the hypotheses, which were drawn from sequence and expression data.

271 **Mapping avirulence genes by para-sexual fusion of different *Fusarium oxysporum* f. sp. *lycopersici* races.**
Hedwich A.S. Teunissen, Jurriaan J. Mes, Jurgo Verkooijen, Ben J.C. Cornelissen and Michel A. Haring. Plant Pathology, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, the Netherlands.

Resistance of tomato to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) is monogenic and dominant. Fol race 1 isolates, containing the avirulence gene 1 *(A-1)*, are avirulent on tomato lines harbouring the corresponding resistance gene *(I-1)*. Fol race 3 isolates contain the avirulence gene 3 *(A-3)* and are therefore avirulent on *I-3* plant lines. For the isolation of either the Fol *A-1* gene and the *A-3* gene a genetic approach was chosen. Since no sexual stage of this fungus is known, para-sexual crosses were used for mapping studies. A race 1 isolate (putative genotype A1a2a3) and a race 3 (a1a2A3) isolate were transformed with either a phleomycin or a hygromycin resistance gene containing plasmid. Transformants with a single insertion that have retained their pathogenic characteristics were selected. Protoplast fusion of a phleomycin resistant race 1 transformant and a hygromycin resistant race 3 transformant resulted in progeny resistant against both antibiotics. 31 Fusionproducts originating from seven different parental combinations were identified. Pathogenicity tests were performed with the stable fusionproducts using tomato lines carrying either an *I-1* or *I-3* resistance gene. 28 Fusionproducts behaved in a race 1-like manner (*A-1* present; *A-3* absent). Only one fusionproduct was able to overcome *I-1* resistance (*A-1* absent; *A-3* present). 2 Fusionproducts showed an "avirulence-recombinant" phenotype suggesting the presence of both *A-1* and *A-3*. Retrotransposon based *"Foxy"*-AFLP analysis showed exchange of parental DNA in all fusionproducts. Based on 100 polymorphisms between race 1 and race 3, the race 1 genetic contribution in the fusionproducts ranged from 26-96%. One interesting polymorphic fragment was identified showing a 100% correlation with the race 1 phenotype. This fragment will be cloned, analysed and used in further finemapping of the Fol avirulence gene *A-1*.

272 **Analysis of the expression of the virulence factor-encoding gene *pksP* of *Aspergillus fumigatus* using the green fluorescent protein**
Kim Langfelder[1], Bruno Philippe[2], Bernhard Jahn[3], Jean-Paul Latg [2], and Axel A. Brakhage[1]. [1]Institut fur Mikrobiologie und Genetik, Technische Universitat Darmstadt, Schnittspahnstrasse 10, D-64287 Darmstadt, F.R.G.; [2]Institut Pasteur, Laboratoire des Aspergillus, 25 Rue du Docteur Roux, F-75015 Paris, France; [3]Institut fur Medizinische Mikrobiologie und Hygiene, Universitat Mainz, D-55101 Mainz, F.R.G.

Aspergillus fumigatus is an important pathogen of the immunocompromised host causing pneumonia and invasive disseminated disease with high mortality. In order to analyse putative virulence factor-encoding genes in vivo, the enhanced green fluorescent protein (EGFP) was established as a reporter. Two promoters of *A. fumigatus* were fused with the *egfp* gene. The promoter of the *pyrG* gene, encoding orotidine-5'-phosphate decarboxylase, and that of the *pksP* gene. *pksP* encodes a polyketide synthase involved in both pigment biosynthesis and virulence. EGFP production was analysed by fluorescence spectrometry, Western blot analysis and fluorescence microscopy. PYRG-EGFP derived fluorescence was detected during all fungal developmental stages. In addition, *pyrG-egfp* expression was detected in germinating conidia when isolated from the lungs of immunosuppressed mice. By contrast, PKSP-EGFP derived fluorescence was found only in phialides and conidia under standard conditions, indicating a developmentally controlled expression of the gene. Interestingly, PKSP-EGFP derived fluorescence was also

detected *in vivo* in hyphae of germinating conidia when isolated from the lungs of immunosuppressed mice. This finding suggests that the *pksP* gene is also expressed in hyphae during invasive growth of the fungus. 1. Jahn *et al.* (1997) IAI **65**:5110 2. Langfelder *et al.* (1998) Med Microbiol Immunol **187**:79 3. Brakhage *et al.* (1999) Contrib to Microbiol, Vol. **2**:205 4. Jahn *et al.* (2000) IAI **68**:3736

273 **Visualization of interactions between *Pseudomonas* biocontrol bacteria and *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomato roots using autofluorescent proteins.** A. Lagopodi[1], G. M. Bloemberg[1], A.F.J. Ram[1,2], A. Wijfjes[1], C.A.M.J.J. van den Hondel[1,2] and E.J.J. Lugtenberg[1]. [1]Leiden University, Inst. Mol. Plant Sciences, Leiden, The Netherlands,[2] Department of Appl. Microbiol. Gene Techn., TNO- Nutrition, Zeist, The Netherlands.

Biological control of soil-borne plant pathogenic fungi with rhizobacteria forms an alternative to the use of chemical pesticides. In order to improve the efficacy of biocontrol fundamental knowledge of the interactions between plants, biocontrol bacteria and phytopathogenic fungi is required. *Pseudomonas fluorescens* WCS365 and *P. chlororaphis* PCL1391 have been shown to efficiently suppress crown and root rot disease of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*F.o.r.l.*) in greenhouse experiments (1, 2). Biocontrol of tomato crown and root rot strains was successfully reproduced in a gnotobiotic sand system after inoculations of tomato seedlings with *F.o.r.l* and the biocontrol *Pseudomonas* strains. In this system where the host, the pathogen and the biocontrol agent are present as the only biotic factors, biocontol can be studied in detail. Recently *F.o.r.l.* was successfully transformed with gfp (green fluorescent protein) and gfp expression was stable after several generations on nutrient media as well as on tomato. At present different autofluorescent proteins are used to mark biocontrol *Pseudomonas* strains. The use of different autofluorescent proteins will give the opportunity to visualize the interactions between the plant, the pythopathogenic fungus and the biocontrol bacterium simultaneously, and to study the nature of the biocontrol effect. Unraveling the interactions of biocontrol bacteria and pathogenic fungi on tomato roots will contribute to development of more efficient methods for biocontrol.

1. Dekkers L.C. 1997. PhD Thesis, Leiden University, The Netherlands. 2. Chin-A-Woeng T. F. C. et al., 1998. Mol. Plant Microbe Interact.11: 1069- 1077.

274 **Involvement of a pacC homolog from *Sclerotinia sclerotiorum* in sclerotial development and virulence.** Jeffrey A. Rollins. University of Florida, Plant Pathology, Gainesville, FL

The broad phytopathogenic potential of *Sclerotinia sclerotiorum* has been attributed to the production and synergistic activities of oxalic acid and cell wall degrading enzymes. The acid environment created by oxalic acid secretion increases hydrolytic enzyme activity, stimulates sclerotial initiation, and inhibits further oxalic acid production. The role of ambient pH in the regulation of these processes at the gene-expression level is being investigated. A homolog of the *Aspergillus nidulans* pH-responsive transcription factor, *pacC*, has been cloned from *S. sclerotiorum*. This gene, *pac1* , can functionally substitute for *pacC* in *A. nidulans*. Expression of *pac1* in *S. sclerotiorum* is dramatically increased under alkaline growth conditions and within aerial hyphae participating in sclerotial development. Targeted disruption of the *pac1* gene was accomplished. Cultures of *pac1* null strains grown without exogenous control of pH were reduced in oxalic acid accumulation by approximately fifty percent. The growth rate and gross hyphal morphology, however, appeared unchanged. These strains were also able to initiate sclerotial morphogenesis, but did not form mature, fully developed sclerotia. Virulence of null *pac1* strains was also reduced. Whether this attenuated virulence is the direct result of reduced oxalic acid production is being investigated. These results indicate that *pac1* is not essential for oxalic acid production but may play a role in regulating the levels of this and possibly other factors necessary for full virulence and sclerotial development. Gene expression and phenotypic characterization of null *pac1* strains in response to varying ambient pH conditions is underway.

275 **A web-based resource for information on vegetative compatibility groups of *Fusarium oxysporum*.** Talma Katan and H. Corby Kistler. The Volcani Center, Bet Dagan, ISRAEL and USDA ARS Cereal Disease Laboratory and Plant Pathology Department, University of Minnesota, St. Paul, USA.

Fusarium oxysporum is a ubiquitous soilborne species that includes saprophytes as well as important plant pathogens. Information on vegetative compatibility (the ability of closely related strains to form heterokaryons in laboratory tests) has been used to study and catagorize genetic diversity in this fungus. As a species, *F. oxysporum* affects over 100 host species, but individual strains are highly host specific. Strains also have been categorized by host specificity into groups known as *formae speciales*. A vegetative compatibility group (VCG) usually includes only strains from a single *forma specialis* whereas *formae speciales* may be comprised of one or more VCG. The correspondence of VCG with host specificity has been useful in assigning strains to host specificity and in estimating the diversity of strains causing disease on a particular host. A numbering system has been established in which *formae speciales* and VCGs have been assigned numerical codes. Each code number is composed of a 3-digit *forma specialis* code and a 1-2 digit serial number for individual VCGs within a *forma specialis*. To date 48 *formae speciales* have been subjected to VCG analysis and the number of VCGs in a *forma specialis* ranged from 1 to 24, averaging four VCGs per *forma specialis*. A web site has been established that compiles information on VCGs of *Fusarium oxysporum* and can be accessed at www.cdl.umn.edu//scab/vcg.html. *Formae speciales* are tabulated by their numerical codes, host plants and the VCGs identified for them. A comprehensive list of 193 references is also included.

276 **Virulence in *Mycosphaerella graminicola* (anamorph *Septoria tritici*) is associated with deletions in a putative avirulence locus.** Gert Kema, Els Verstappen, Odette Mendes, Ineke de Vries and Cees Waalwijk. Plant Research International, Wageningen, The Netherlands

Gene-for-gene interactions may play a role in structuring *Mycosphaerella graminicola* populations. We generated segregating populations by crossing two Dutch field isolates, IPO323 (avirulent) and IPO94269 (virulent) and studied F1, BC1 and F2 populations by inoculation assays on five wheat cultivars. Avirulence inherited as a monogenic character. A genetic linkage map was generated and the putative *avr* locus as well as three co-segregating AFLP markers were mapped. These markers were used to select specific BAC clones from both parents. Sequence analyses of corresponding contigs of both parents revealed an ORF coding for a putative protein carrying a signal peptide that was absent in the virulent parent. The flanking regions of this 840 bp deletion showed a strong homology, which enabled us to design primers that produced either a 1500 bp or a 650 bp fragment. The frequency of this deletion in several natural *M. graminicola* populations ranged from 80 to 95%. This suggests that the deletion occurred only once, which is unexpected if this locus would be under selection. The frequency of avirulent phenotypes in natural populations not always correlated with the presence of the ORF, which is an indication that more cultivar specific avirulence loci may play a role in this pathosystem. The functional analysis of this *avr* locus is under way.

277 **EST data mining: Novel extracellular proteins from the oomycete plant pathogen *Phytophthora infestans.*** Trudy Torto, Allison Styer, Sophien Kamoun. Department of Plant Pathology, The Ohio State University-OARDC,Wooster, OH

Interactions between plants and microbial pathogens involve complex signal exchanges at the plant surface and intercellular space interface. Surface components of a pathogen must play important roles in the development of a complete infection cycle and recognition by resistant plants. We aim at identifying extracellular proteins from*Phytophthora infestans*, an economically important oomycete pathogen. Targeting extracellular proteins will increase the probability of identifying proteins essential for virulence and survival of the pathogen. We developed and validated an algorithm (PexFinder V1.0) for automated identification of secreted and membrane proteins from expressed sequence tag (EST) data sets. The program integrates a series of sequence analysis scripts with signal peptide predictions based on SignalP V2.0 (http://www.cbs.dtu.dk/services/SignalP-2.0/). Analysis of 2,147 ESTs from *P. infestans* using PexFinderidentified 261 ESTs (12.2%) corresponding to a set of 145 nonredundant Pex (*Phytophthora* extracellular proteins) genes. Of these, 85 (59%) Pex genes are novel with no significant matches in public databases. The algorithm was validated using a number of methods. For example, PexFinder identified numerous genes with significant matches to known extracellular proteins, as well as all previously characterized extracellular proteins from *Phytophthora* that were represented in the EST data set. Functional genetic assays, such as high throughput virus and *Agrobacterium*-based expression systems, are being applied to the novel Pex genes to determine their role in virulence/avirulence. This approach led to the discovery of novel classes of extracellular signal molecules from

Web: http://www.oardc.ohio-state.edu/phytophthora

278 **Comparison of compatible interactions between tomato and *Phytophthora infestans*.** Christine D. Smart[1], Kevin L. Myers[1], Mark D. D Ascenzo[2], Paul P. Debbie[2,3], Gregory B. Martin[1,2], and William E. Fry[1]. [1]Department of Plant Pathology, Cornell University, [2]The Boyce Thompson Institute at Cornell University, [3]BTI Center for Gene Expression Profiling at Cornell University, Ithaca, NY

Tomato-specialization in the oomycete pathogen *Phytophthora infestans* is characterized by a biotrophic growth habit and increased pathogenicity on tomato. To investigate the mechanism of this specialization, we compared tomato host responses to a specialized isolate, to those of a non-specialized (more necrotrophic and less aggressive) isolate. As expected, we saw induction of the hypersensitive response (HR) more quickly in tomato plants inoculated with the non-specialized isolate. Induction of pathogenesis-related (PR) genes known to be markers for the salicylic acid or jasmonic acid signaling pathway was similar in plants inoculated with specialized or non-specialized isolates. However, basic PR genes known be ethylene-regulated were induced more quickly in tomato plants inoculated with the specialized (biotrophic, more aggressive) isolate than in plants inoculated with the non-specialized isolate. These findings support the hypothesis that while HR is necessary for a reduction in aggressiveness, induction of the 10 PR genes tested is not responsible for the difference between highly pathogenic (tomato-specialized) and moderately pathogenic (non-specialized) isolates of *P. infestans*. We are currently using microarray analysis to identify differences in gene expression between these two compatible interactions.

279 **Identification of differentially expressed genes involved in the symbiosis between *Neotyphodium coenophialum* and tall fescue by suppression subtractive hybridization analysis.** L.J. Johnson[1], R.D. Johnson[1], C.L. Schardl[2], and D.G. Panaccione[1]. 1, West Virginia University, Morgantown; 2, University of Kentucky, Lexington.

Tall fescue (*Festuca arundinaceae*) is typically infected with *Neotyphodium coenophialum*, an asexual, endophytic fungus. Colonization is completely internal in host tissue with no visible symptoms, suggesting that the plant does not respond to endophyte colonization. This fungal endophyte provides many benefits to its plant host, such as increased biomass and seed production, protection from insects, drought tolerance and resistance to nematodes and some fungal pathogens. We hypothesize that the underlying physiology of the plant-endophyte interaction is the result of changes in gene expression. Our research focuses on identifying genes that have altered expression in the plant/fungal symbiosis. Using suppression subtractive hybridization (SSH), a PCR-based cDNA subtraction technique, we have currently identified at least 23 differentially expressed genes. Up-regulated plant genes of particular interest encoded a PDR5-like ABC transporter, an Omega-3 fatty acid desaturase, and a heat shock protein (hsp70). In addition, genes suppressed in the plant due to the presence of endophyte were also identified. These included genes for an aminopeptidase N, a chlorophyll A-B binding protein, methionine associated enzymes, and a pathogenesis-related protein (PR- 10). Interestingly, several of these suppressed plant genes have been reported to be induced in other plants by fungal pathogens. Overall these results suggest that tall fescue is an active participant in the endophyte symbiosis and that the endophyte may be suppressing plant defense genes. Further analysis and identification of genes with altered expression in the plant fungus symbiosis may elucidate mechanisms responsible for the benefits associated with endophyte infection of tall fescue.

280 **Characterization of an ABC transporter from *Phytophthora sojae***. Paul F. Morris, Mary S.Connolly. Bowling Green State University, Biological Sciences, Bowling Green, OH, USA

ABC transporters are members of a large protein super family involved in both the uptake and efflux of compounds across membranes. These transporters have been identified in both prokaryotic and eukaryotic organisms. A 1300bp fragment from the 3' end of ABC transporter has been identified in both zoospores and *Phytophthora. sojae* - infected soybean hypocotyls. Sequence analysis suggests that this gene is part of the pleiotropic drug resistance family of ABC transporters, many of which are up-regulated by compounds that also serve as substrates for the transporter. Homologues of the *Phytophthora* gene are present in *Arabidopsis* and *Spirodella*, and the *Spirodella* gene is known to be up-regulated in response to cold temperatures, osmotic stress, and abscisic acid. The response of the *P. sojae* gene to a variety of environmental stimuli will be presented.

281 **Detection of plant genes activated by *Ustilago maydis* infection in an alternate host**. Cristina G. Reynaga-Peña[1], Jos Ruiz-Herrera[1] and Patricia S. Springer[2] . [1] Depto. de Ingenieria Genética, CINVESTAV Unidad Irapuato, Irapuato, Gto., México and [2] Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA, U.S.A.

In this work we are using a novel approach towards the understanding of molecular signals involved in plant-pathogen interactions. It is known that *Ustilago maydis*, the pathogenic fungus responsible for corn smut, has a complex life cycle that can only be completed upon invasion of the host plant. It has been proposed that a close relationship between plant and pathogen must exist during *Ustilago* infection, where communication between both organisms is necessary. Previously, our lab has started to use tissue cultures and alternative host plants to study *Ustilago*-plant interactions. We are now characterizing a novel infection model system using *Arabidopsis thaliana* as a host plant, and have observed mycelial growth of *Ustilago* on several plant tissues. The development of this model system permitted us the use of an enhancer/gene trap transposant system that has been developed in *Arabidopsis* to identify genes that show changes in expression during Ustilago infection. At this time, preliminary results indicate that several transposants show differential expression of the reporter gene upon infection. These transposants are now in the process of identification.

282 **Identification of genes involved in pathogenicity of the fungal common bean pathogen *Colletotrichum lindemuthianum.*** R. Lauge , D. Parisot, A.-L. Pellier, C. Veneault, M. Dufresne and T. Langin. Laboratoire de Phytopathologie Moléculaire, Institut de Biotechnologies des Plantes, batiment 630, Université Paris-Sud, 91405 Orsay Cedex, France.

Insertional mutagenesis using the plasmid pAN7-1 was done on the fungal pathogen *Colletotrichum lindemuthianum*. 1100 independant transformants were tested for impaired pathogenicity on common bean. 11 mutants were identified, of which most are blocked during the early stages of the infection cycle: at the penetration step or during the biotrophic phase. A fine cytological characterization of the phenotype and the level of blockage of the various mutants is underway. Molecular analyses and demonstration of the link between the insertion event and the pathogenicity phenotype have been achieved for three mutants and all of them are tagged. The inability of two mutants to penetrate (although they produce appressoria) is due to disruption of genes encoding a serine/threonine kinase (*clk1*) and a copper ATPase transporter (*clap1*) respectively. We are currently investigating the precise role of these two genes in appressorium functionality. A third mutant is blocked at the end of the biotrophic phase after the differentiation of the infection vesicle and of the primary hyphae. The disrupted gene (*clta1*) encodes a putative transcriptional activator of the GAL4 family. A search for the sequences that are under the control of this activator has been initiated using a one-hybrid derived strategy. The genes that will be obtained should provide us with information on the type of function that is regulated by CLTA1. Of the remaining mutants, six that display a clear phenotype are under molecular analysis. Preliminary results show that insertion flanking sequences display strong similarities with a hexose transporter and a regulator of inorganic phosphate transport for at least two of these mutants. Latest data will be presented and discussed.

283 **Characterization of the *ToxB* gene from *Pyrenophora tritici-repentis***. J. Patrick Martinez, Sean A. Ottum, and Lynda M. Ciuffetti. Department of Botany and Plant Pathology. Oregon State University, Corvallis 97331 U.S.A.

Race 5 isolates of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat, are characterized by the production of lesions with spreading chlorosis on wheat cultivar Katepwa. This host-specific chlorosis has been correlated with a 6.61 kDa host-specific toxin (HST), Ptr ToxB (Strelkov et al., 1999, MPMI 12:728-732). A 261 bp gene, *ToxB*, was cloned and characterized from a race 5 isolate of *P. tritici-repentis*. *ToxB* encodes a putative 23 amino acid signal peptide and a 64 amino acid HST, Ptr ToxB. The *ToxB* gene appears to be present in multiple copies in race 5 isolates. Non-pathogenic isolates of this fungus contain a single copy gene (*toxb*) that shows 86 % identity at the nucleotide level when compared to *ToxB*. Analysis of Ptr ToxB from heterologous expression in *Pichia pastoris* confirms that *ToxB* encodes a HST. Current research will attempt to determine the function of these "additional" *ToxB* genes, and their relationship to the *toxb* gene in non-pathogenic isolates.

284 **Transformation of *Pythium aphanidermatum* to geneticin resistance.** John J. Weiland. Sugarbeet and Potato Research Unit, USDA-Agricultural Research Service, Northern Crop Science Laboratory, Fargo, N.D. 58105-5677

Conditions for the production of protoplasts and gene transfer in *Pythium aphanidermatum* were investigated. Efficient protoplast generation was possible after culture of mycelium in potato dextrose broth followed by digestion with 0.5% (w/v) each of cellulase and beta-D-glucanase. Plasmid pHAMT35N/SK encoding the *nptII* gene under control of the Ham34 promoter from the oomycete *Bremia lactucae* was used to define electroporation parameters for gene transfer. A square-wave electroporation pulse of 2500V/cm at 50 microfarad capacitance reproducibly produced transformants, albeit at low efficiency (0.1-0.4 transformants from ~100,000 regenerable protoplasts per microgram of DNA). Twenty seven independant transformants exhibited wild-type growth on potato dextrose agar amended with geneticin at 50 microgram per ml, a concentration that near completely inhibited the growth of untransformed fungus. Southern blot analysis indicated that transforming DNA was integrated into the fungal genome as a tandem array of plasmid monomers. Co-electroporation of of pHAMT35N/SK with pEGFP encoding enhanced green fluorescent protein (EGFP) under the control of the immediate early promoter from the mammalian cytomegalovirus produced transient expression of blue-green fluorescence. Application of the technique to studies on the biochemical basis for pathogenesis in this agriculturally-important group of fungi are discussed.

285 **Multidrug resistance proteins of *Cochliobolus heterostrophus.*** Uvini P. Gunawardena, Olen C Yoder, Gillian Turgeon. NADII, Plant Health, San Diego, CA

Multi drug resistance (MDR) proteins in plant pathogenic fungi are of interest for their potential to mediate interactions between hosts and pathogens, as well as for the opportunities they offer to genetically engineer plants for resistance to microbial virulence factors such as mycotoxins. In plant pathogenic fungi, two major groups of MDR proteins, ABC (ATP Binding Cassette) and MFS (Major Facilitator Superfamily) transport proteins, have been shown to play important roles during fungal interactions with host plants. We have mined the genomic sequence of *Cochliobolus heterostrophus*, a pathogen of corn, and found 51 putative ABC and more than 20 putative MFS proteins. The genes encoding these proteins are being targeted for deletion and the resulting mutants tested for viability for alteration of their virulence to corn, and for their sensitivity to plant antimicrobial compounds.

286 **Calcineurin (CN) affects morphology, mating and pathogenicity in *Ustilago maydis* .** J. Duick Egan and S.E. Gold. Dept. of Plant Pathology, Univ. of Georgia, Athens, GA 30602.

Ustilago maydis is a dimorphic basidiomycete and the causal agent of corn smut disease. Dimorphism is controlled through signal transduction pathways involving cAMP and the mitogen-activated protein (MAP) kinase cascade. Protein phosphatases reverse the effects of protein kinase phosphorylation. Calcineurin (CN, also known as protein phosphatase 2B, PP2B) is a serine/threonine protein phosphatase and, in other systems, has a role in reversing the phosphorylation of the substrates of cAMP-dependent protein kinase. Therefore, we hypothesize that CN plays an important role in dimorphism, and mutants would have enhance budding growth. Mutants in the *U. maydis* CN catalytic subunit, ucn1, are viable and indeed have a multiple budding phenotype. Mating reactions and pathogenicity of these mutants are severely reduced and galls never developed in inoculated maize plants. These results suggest that CN plays an important role in morphogenesis, mating, and pathogenicity in *U. maydis*.

287 **Progress towards cloning avirulence genes in the wheat stem rust fungus, *Puccinia graminis*.** Les J. Szabo and Alexi Balmuth. Cereal Disease Laboratory, ARS, USDA, St. Paul, Minnesota, U.S.A.

Puccinia graminis is a heteroecious rust fungus, with uredinial (asexual) and telial stages on cereal and forage grass species and the pycnial and aecial stages on members of the barberry family. Genetics has demonstrated that infection of wheat by *P. graminis* f.sp. *tritici* follows a gene-for-gene interaction involving resistance genes in the host and avirulence genes in the rust pathogen. In order to better understand this interaction, we have undertaken the cloning of several avirulence genes from *P. graminis* f.sp. *tritici*. A mapping population of *P. graminis* has been developed in which eight single dominant avirulence genes are segregating (Zambino et.al., 2000). DNA markers (AFLPs and RAPDs) have been used to construct a partial genetic map in which these avirulence genes have been mapped to seven linkage groups. Cloning and characterization of DNA markers linked to avirulence genes will be presented.

288 **A Class I hydrophobin from *Cochliobolus heterostrophus.*** Scott E. Baker, Olen Yoder and Gillian Turgeon. Plant Health Department, Novartis Agricultural Discovery Institute, 3115 Merryfield Row, San Diego, CA, 92121

A gene encoding a class I hydrophobin *(CHH1)* was identified in the corn pathogen *Cochliobolus heterostrophus. CHH1* encodes a putative protein (CHH1p) of 116 amino acids that has 31% identity with the *Magnaporthe grisea MPG1* protein, also a class I hydrophobin. We have generated deletions of *CHH1* and our phenotypic analysis indicates that appressorium formation, conidiation and virulence are unaffected by loss of this protein, in contrast to *MPG1* deletions which show reduced conidiation, appressorium formation and virulence. These data are consistent with the prior observation that neither melanin production nor appressoria are necessary for full virulence of *C. heterostrophus*. *M. grisea* requires both melanin and functional appressoria for infectivity. Moreover, a G alpha protein (CGA1 in *C. heterostrophus*, MAGB in M. grisea) is important for infection by *M. grisea* but not *C. heterostrophus* (Horwitz et al. FG&B 1999 26:19).

289 **Cloning of a functional avirulence gene homolog from a *Magnaporthe grisea* strain that is a pathogen of perennial ryegrass**. Rebecca Peyyala and Mark Farman. Department of Plant Pathology, University of Kentucky, USA

Magnaporthe grisea (anamorph = *Pyricularia grisea*) causes a serious disease of perennial ryegrass (*Lolium perenne*) called gray leaf spot. Perennial ryegrass appears to have little or no natural resistance to this disease. Rice is resistant to strains of *M. grisea* that cause gray leaf spot indicating that it may be a good source of resistance to this disease. Southern hybridization analysis of *M. grisea* strains infecting perennial ryegrass revealed that they have a homolog of the *AVR1-CO39* avirulence gene, which prevents *M. grisea* from infecting rice cultivar CO39 due to the presence of the corresponding resistance gene *Pi-CO39(t)*. The homolog, which we refer to as *AVR1-CO39^{Lp}*, was amplified by PCR, cloned and sequenced. It differed from *AVR1-CO39* by three point mutations and 2 frame shifts but these did appear to affect the presumed *AVR1-CO39* transcription unit. The *AVR1-CO39^{Lp}* gene was then transformed into fungal strain ML33-2 that is virulent on CO39. Transformants were tested for the presence of *AVR1-CO39^{Lp}* by Southern hybridization and the function of the gene was tested by inoculation on CO39. ML33-2 transformants carrying *AVR1-CO39^{Lp}* were avirulent on CO39 but were unaffected in their ability to infect 51583, a cultivar that lacks *Pi-CO39(t)*. Therefore, *AVR1-CO39^{Lp}* is a functional avirulence gene that likely prevents the perennial ryegrass pathogens from infecting CO39 rice, due to recognition by the *Pi-CO39(t)* resistance gene. Experiments are in progress to determine if *Pi-CO39(t)* will also function in perennial ryegrass to confer resistance to gray leaf spot disease.

290 **Identification of a Cys(6)-Zn(2) transcription factor that binds the pisatin-responsive region of *PDA*1 in *Nectria haematococca*.** Rana Khan, Reynold Tan and David Straney. Dept. Cell Biol. & Molec. Genetics, University of Maryland, College Park, MD. 20742

The pea pathogen *Nectria haematococca* MP VI (*Fusarium solani*) provides a genetic model system for soil borne pathogens. One of the intriguing aspects of the interaction of this fungus with its host, garden pea (*Pisum sativum*), is its use of host-specific compounds to coordinate both developmental and gene-specific responses in pathogenesis. Pisatin, the isoflavonoid phytoalexin produced by pea, provides a host-specific cue for both of these responses. Expression of the *PDA*1 gene, which encodes a cytochrome P450, is highly induced by exposure of mycelium to pisatin. Pisatin appears to be the major inducer for this gene during pathogenesis as well. PDA1 regulation provides a system to identify fungal components that allow a pathogen to recognize and respond to host-specific cues to coordinate pathogenesis. We have defined a 40 bp region in the *PDA*1 promoter that mediates pisatin-responsive transcription in vivo. A DNA-binding factor was found to bind this 40 bp region in mycelial extracts. The yeast one-hybrid system was used to clone the gene encoding the protein which binds this 40 bp region. Sequence motifs from the cloned gene suggest that it is a Cys(6)-Zn(2) binuclear cluster factor. The cloning of this factor will allow testing the possibility that it may bind pisatin as well and so function as a nuclear receptor, similar to the mechanism regulating many mammalian cytochrome P450 genes.

291 **High frequency mitotic gene conversion across the genome of the oomycete , *Phytophthora sojae*.** Jureerat Chamnanpunt and Brett M Tyler. Department of Plant Pathology, University of California, Davis,USA.

Microbial populations depend on genetic variation to respond to novel environmental challenges. Plant pathogens are notorious for their ability to overcome pesticides and host resistance genes as a result of genetic changes. We show here that in *Phytophthora sojae* , an oomycete pathogen of soybean, high frequency of mitotic gene conversion rapidly converts heterozygous loci to homozygosity, resulting in heterokaryons containing a highly diverse

population of nuclei. We examined gene conversion within a selected linkage group, and in unlinked markers throughout the genome. Loci as close as 0.7 kb apart underwent independent conversion at frequencies of up to 80%. In many cases, conversion was highly polar, proceeding in only one direction, suggesting that conversion was initiated by allele-specific double stranded breaks. High frequency conversions were initiated when certain strains of *P.sojae* were mated to produce F1 hybrids. They continued throughout vegetative growth and were stimulated by further sexual reproduction. Pedigree analysis indicated that individual loci undergo multiple independent conversions within the nuclei of a vegetative clone, and that conversion may be preceded by a heritable "activation" state that commits the direction of conversion. The high polarity of gene conversion was determined in *cis* by the sequence polymorphism at the site of conversion. The conversion frequency is strongly affected by genetic background of some particular strain (P7076). We proposed the hypothesis that the double stranded breaks were stimulated by P7076 genome or interaction with P7076 genome.

292 **Maize ribosome-inactivating protein has antifungal activity against Aspergillus species.** Kirsten Nielsen and Gary A. Payne. NC State University, Plant Pathology, Raleigh, NC

The abundant maize kernel ribosome-inactivating protein (RIP) was tested for antifungal activity against *Aspergillus nidulans* and *Aspergillus flavus*. A striking decrease in hyphal proliferation was observed when conidia of *A. nidulans* were treated with RIP protein. RIP treatment of conidia from *A. flavus* resulted in increased hyphal branching. These results indicate that both fungi were affected by RIP treatment but the toxicity seen with treatment of *A. nidulans* is apparently avoided by *A. flavus*. Even though conidia were treated with RIP prior to germination, a developmental time-course revealed that changes in fungal growth for both species were not seen until the post-divisional growth stage. The inhibitory activity of RIP against normal fungal growth is consistent with a biological function to protect the seed from fungal invasion. To determine whether or not the antifungal effect of RIP was due to its enzymatic activity, we constructed expression plasmids with point mutations at active site residues. The resulting proteins had no effect on the growth of *A. nidulans* and no ribosome-inactivating activity in vitro. These results indicate that the antifungal activity of RIP requires the enzymatic ribosome- inactivating activity. However, these results also lead to the question ûû How does RIP1 enter the fungal cell to inactivate the ribosomes? At 25 kD, maize RIP would appear to be too large to cross the fungal cell membrane. We are investigating the means by which RIP exerts its antifungal effect by localization of fluorescently labeled RIP or control proteins during their interaction with *A. nidulans*.

293 **Characterizing the PMK1 Pathway in *Magnaporthe grisea*.** Jin-Rong Xu, Li Zheng, Marie Nishimura, Gyungsoon Park, Chaoyang Xue, Stephen Lam. Purdue University, Botany & Plant Pathology.

Rice blast, caused by *Magnaporthe grisea*, is one of the most severe fungal diseases on rice throughout the world. Like many other fungal pathogens, *M. grisea* develops specialized structures (appressoria) to invade its hosts. We have identified a MAP kinase PMK1 (Pathogenicity MAP kinase 1) that is essential for appressorium formation and infectious growth in *M. grisea*. To understand how the PMK1 MAP kinase is activated, we have isolated several putative components of the PMK1 pathway including homologues of budding yeast Ste4, Ste20, Ste11 and Ste12. Gene replacement mutants of these *M. grisea* genes will be isolated to determine their roles in the PMK1 signal transduction pathway. A yeast two-hybrid library has been constructed to identify genes interacting with PMK1. Data from the preliminary screening will be presented. In addition, we have constructed a subtractive library enriched for genes regulated by PMK1. Three genes identified in this library have been selected for further characterization.

294 **Development of selectable markers in the human-pathogenic fungus *Histoplasma capsulatum*.** Adam K. Bahrami and Anita Sil. Department of Microbiology & Immunology, University of California-San Francisco

Genetic approaches to studying the human-pathogenic fungus *Histoplasma capsulatum* are limited by the lack of sufficient marker genes to select for the uptake of transforming DNA. A *H. capsulatum* cDNA library was screened for clones that complement auxotrophic mutations of *Saccharomyces cerevisiae*. We identified cDNA clones that complement *S. cerevisiae* ade2, leu2, or his3 mutations and are now disrupting the corresponding genomic loci. Additional work aims to identify a conditional promoter for use in the study of this organism.

295 **Analyses of a ketoreductase ORF at the AVR1-MARA locus of *Magnaporthe grisea.*** Michael W. Harding, M. Alejandra Mandel and Marc J. Orbach. Department of Plant Pathology, University of Arizona, Tucson, AZ.

We are working to characterize the gene AVR1-MARA from the rice blast fungus *Magnaporthe grisea*. AVR1-MARA is a stable avirulence gene that segregates as a single locus in strain 4224-7-8, conditioning an avirulent response on the rice cultivar Maratelli. A map-based cloning approach has been taken to clone AVR1-MARA. An open reading frame with significant homology to a ketoreductase gene from *Streptomyces hygroscopicus* was found in DNA sequence linked to this locus. The ketoreductase in *S. hygroscopicus* is part of the rapamycin polyketide biosynthetic cluster. We are examining expression of the ketoreductase mRNA under a variety of growth conditions. Expression is also being examined in infected rice plants. Total RNA from *M. grisea* cultured in media with complete or limiting nutritional components or infected rice leaves will be isolated and analyzed by northern analysis. To determine whether ketoreductase function is necessary for avirulence, we are currently working to disrupt the gene by targeted gene replacement. The ketoreductase in 4224-7-8 will be replaced with a selectable resistance marker and the mutant strain tested for virulence on 'Maratelli'. The current data on ketoreductase expression and function will be discussed.

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Gene Regulation

296 **Analysis of the function and regulation of the NADP-isocitrate dehydrogenase (NADP-IDH) gene from *Aspergillus nidulans.*** Edyta Szewczyk, Sophie Kourambas, Meryl A Davis and Michael J Hynes. University of Melbourne, Department of Genetics, Parkville, Australia

Many micro-organisms can use two-carbon compounds, like acetate or ethanol, as well as long chain fatty acids as sole carbon sources. Pathways and enzymes of the glyoxalate bypass, of fatty acid beta-oxidation, located in peroxisomes, as well as of the citric acid cycle located in mitochondria are required for the utilization of these carbon sources. The C6 zinc cluster protein FacB is required for acetate induction of enzymes involved directly in acetate metabolism, as well as of the glyoxalate bypass enzymes. We have previously shown that NADP-dependent isocitrate dehydrogenase activity is also regulated by FacB dependent acetate induction and by CreA mediated carbon catabolite repression in a similar manner to *amdS*, *facA, acuD* and *acuE*.

The structural gene (*idpA*) has been cloned and shown to be regulated by CreA and FacB and also by induction by fatty acids. The predicted protein possesses both an N-terminal mitochondrial targeting signal and a C-terminal peroxisomal signal. This provides an interesting contrast with the situation in *Saccharomyces cerevisiae* where there are three genes encoding enzymes located in separate cell compartments. By the use of fluorescent protein (GFP, RFP and BFP) fusion constructs, *idpA* encoded NADP-isocitrate dehydrogenase has been found to be present in peroxisomes and mitochondria. The structure of the gene suggests the presence of two alternative start points of transcription that may result in the two different forms of the enzyme.

No mutants lacking NADP-IDH are known in *A.nidulans*. It is likely that NADP-IDH is required for NADPH generation during growth on acetate and fatty acids and that a deletion of the gene will affect growth on these carbon sources at least to some extent.

297 **Photoinduction of phr1 expression and sporulation is independent in *Trichoderma harzianum.*** Teresa Rosales Saavedra[1], Victor Rocha Ramirez[1], Benjamin A. Horwitz[2] and Alfredo Herrera Estrella[1]. [1]Department of Plant Genetic Engineering, Centro de Investigacion y Estudios Avanzados del I.P.N., Unidad Irapuato, Irapuato, Gto. 36500, Mexico and [2]Department of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel

Blue light and development regulate the expression of the phr1 gene, which encodes the DNA repair enzyme photolyase of the filamentous fungus *Trichoderma harzianum.* Trichoderma also sporulates in a synchronized manner following a brief pulse of blue light. The effect of a set of chemicals known to either stimulate or inhibit the activity of different elements in signal transduction pathways was tested in terms or their effect on photolyase gene expression and conidiation, both under light and dark conditions. dbcAMP bypasses the requirement of light for sporulation, while atropine prevents sporulation even after photoinduction. Light regulation of phr1 however, is indifferent to both these effectors. Induction of photolyase expression behaves as a direct, rapid response to light, independent of the induction of sporulation. Photoinduction of phr1 and light induced sporulation,could be expected to share elements common to signal trandsduction pathways if not the same pathway. Nevertheless, the analysis of the responses with mutants, wich have been altered in the alpha subunit of the heterotrimeric (Tga1) G protein, constitutively activated that in turn inhibited completely their conidiation, allowed us to conclude that sporulation and photolyase expression are distinct in their photoreceptor system or in the transduction of the blue light signal.

298 **Expression of the mycoparasitism related genes prb1 and ech42 in Trichoderma harzianum is determined by two groups of protein kinases.** A. Mendoza-Mendoza; M. Ramos-Vega and A. Herrera-Estrella. Departamento de Ingenieria Genetica de Plantas. Cinvestav-IPN Unidad Irapuato. Guanajuato, Mexico.

Trichoderma harzianum has an antagonic ability towards a phytopathogenic fungi. The mechanisms that confer this capacity are: antibiotic production for competition nutrients and mycoparasitism. The latter involves the production of lytic enzymes that degrade the host cell wall. The prb1 and ech42 genes encode two lytic enzymes produced by *T. harzianum* in response to host or nutrient deprivation, mainly the lack of glucose and nitrogen source. Our results suggest that the expression of these genes in nutrient deprivation depends on the activation of a MAP kinase pathway. Likewise confrontation between *T. harzianum* with *Sclerotium rolfssi* or *Rhizoctonia solani* shows an inverse correlation between the prb1 and ech42 expression and the PKA activity. We have observed that the cAMP levels are modificated when *T. harzianum* is confronted with *S. rolfssi*. All these results suggest that the regulation of expression of prb1 and ech42 in *T. harzianum* is complex and that at least two groups of kinases are involved, the MAP kinase and the cyclic AMP dependent-kinase.

299 **Characterization of the transcription factor PACC and its involvement in cephalosporin C biosynthesis gene regulation in *Acremonium chrysogenum*.** Esther Schmitt, Renate Kempken. Ruhr-Universitat Bochum, Allg. und Mol. Botanik, Bochum, Germany

The analysis of the *pcbC* promoter from the cephalosporin C producing filamentous fungus *Acremonium chrysogenum* was performed using fungal transformants carrying reporter gene fusions. By investigating sequential deletion derivatives of the *pcbC* promoter region, a DNA fragment was identified, which is responsible for transcriptional activation of the *pcbC* gene. Sequence analysis of this fragment revealed a consensus binding site for the fungal PACC transcription factor. Gel retardation experiments with *A. chrysogenum* protein crude extracts confirmed the specific binding of a protein to the PACC binding site. The subsequent cloning of the *pacC* homologue from *A. chrysogenum* allowed the identification of an open reading frame of 621 amino acids encoded by four exons. The polypeptide shows about 35 % identical amino acid positions when compared with other fungal PACC proteins. An *E. coli* synthesized PACC protein fragment was used for *in vitro* binding assays and specific binding of the zinc finger transcription factor to its consensus binding sites in the promoter regions of four cephalosporin C biosynthesis genes could be demonstrated. The bi-directional promoters of the *pcbAB-pcbC* and *cefEF-cefG* genes contain two PACC binding sites each. The obtained data strongly suggest that in *A. chrysogenum*, the zinc finger transcription factor PACC is involved in the transcriptional regulation of the cephalosporin C pathway genes.

Schmitt EK, Kempken R, Kuck U. Functional analysis of promoter sequences of cephalosporin C biosynthesis genes from *Acremonium chrysogenum*: specific DNA-protein interactions and characterization of the transcription factor PACC. Mol Gen Genet (submitted)

300 **The CPCR1 transcription factor is important for timely expression of a cephalosporin C biosynthesis gene in *Acremonium chrysogenum*.** Esther K. Schmitt and Ulrich Kück. Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Universität Bochum, 44780 Bochum, Germany

The filamentous fungus *Acremonium chrysogenum* is used industrially to produce the -lactam antibiotic cephalosporin C. Our aim is to isolate and characterize transcription factors from *A. chrysogenum* which regulate transcription initiation or repression of biosynthesis genes. The CPCR1 protein was identified in a one-hybrid screen using a sequence from the *pcbAB-pcbC* promoter as a bait. The isolated DNA-binding protein belongs to the conserved family of eukaryotic RFX transcription factors (1). Using a recombinant CPCR1 protein putative binding sites in the two promoter regions of cephalosporin C biosynthesis genes *pcbAB-pcbC* and *cefEF-cefG* were analyzed for interaction with the transcription factor. It was shown that CPCR1 recognizes and binds, in addition to the previously determined binding site, another sequence in the intergenic region between the *pcbAB-pcbC* genes. We also present results from *Acremonium* transformants with multiple copies of the *cpcR1* gene and knock-out strains. These experiments give insight into the regulatory role of CPCR1 with regard to expression of the *pcbC* gene. Fungal transformants were analyzed using northern and western blots and HPLC analysis of culture broth to determine changes in the biosynthesis of cephalosporin C. Together with two other transcription factors that are involved in the regulation of cephalosporin C biosynthesis, namely CRE1 (2,3) and PACC (4), the functional analysis of these regulatory proteins in *A. chrysogenum* will widen our understanding of the molecular regulation of cephalosporin C biosynthesis.

1) Schmitt EK, Kück U (2000) The fungal CPCR1 protein, which binds specifically to B-lactam biosynthesis genes, is related to human RFX transcription factors. J Biol Chem 275:9348-9357 2) Jekosch K, Kück U (2000) Glucose dependent transcriptional expression of the cre1 gene in Acremonium chrysogenum strains showing different levels of cephalosporin C production. Curr Genet 37:388-395 3) Jekosch K, Kück U (2000) Glucose repression is lost in an Acremonium chrysogenum B-lactam producer strain and can be restored by multiple copies of the cre1 gene. Appl Microbiol Biotechnol 54:556-563 4) Schmitt EK, Kempken R, Kück U Functional analysis of promoter sequences of cephalosporin C biosynthesis genes from Acremonium chrysogenum: specific DNA-protein interactions and characterization of the transcription factor PACC. Mol Gen Genet (submitted)

301 **Isolation and characterization of a Vacuolar-ATPase (MVP1) gene of *Magnaporthe grisea***. Frederick O. Asiegbu[1,3], Woobong Choi[1,2], Jun Seop Jeong [1,2] and Ralph Dean[1,2*]. [1]Clemson University Genomics Institute (CUGI), Clemson SC, USA. [2]Fungal Genomics Laboratory, Department of Plant Pathology, North Carolina State University, NC, USA. [3]Dept. of Forest Mycology & Pathology, Swedish University of Agriculture, Uppsala, Sweden.

Vacuolar ATPases play crucial roles in regulating the metabolic activities in the vacuoles of eukaryotic cells. We isolated a gene encoding v-ATPase homologue of *Magnaporthe grisea*, named *MVP1,* from an appressoria cDNA library. The transcript of ~1.6kb contains 546bp of coding sequence with a 3' untranslated region of about 168 bp long and 5' untranslated region of about 870bp long. The hybridization pattern on Southern blots of genomic DNA following digestion with three restriction enzymes (*BamH1, EcoR1,HindIII*) indicated that the gene exists as a single copy *M. grisea* genome. RNA gel blot analyses showed that *MVP1* was highly expressed in germinating spore and mycelial stage compared to appressoria or non germinated spores. *MVP1* showed a high degree of homology to other v-ATPases, particularly with the gene from *Neurospora crassa*. Homologous recombination using a knockout construct in pAN7-1 is being used to disrupt *MVP1*. The pathological and other phenotypic consequences of gene disruption will be presented.

302 **A genetic selection for circadian clock mutations in *Neurospora crassa***. Deborah Bell-Pedersen* and Irene March. Department of Biology, Texas A&M University, College Station, TX 77843

To identify components of the circadian clock system in *Neurospora crassa*, we have carried out a genetic selection to isolate mutations that alter the expression of *clock-controlled genes* (*ccgs*). This selection is based on the differential expression of the ccgs in response to the presence or absence of the clock gene product FRQ. For example, *ccg-1* expression is repressed and *ccg-2* expression is activated when FRQ is present in the cell. The promoter region of the *ccg-1* gene was ligated to *mtr* (1). The *mtr* gene encodes a neutral amino acid permease that allows both positive and negative selection. Loss of function can be selected based on resistance to the amino acid analog p-flourophenylalanine (FPA). Gain of function can be selected based on growth of tryptophan auxotrophs on high arginine/low tryptophan media. The fusion constructs were transformed into both *bd;mtr;trp- 2* and *bd;frq10;mtr;trp-2* strains and homokaryons were isolated. Reconstruction experiments using the *ccg-1:mtr* transformants showed FRQ- dependent production of the amino acid permease: growth of the FRQ+ transformant

on FPA but no growth on tryptophan, and growth of the FRQ-null transformant on tryptophan but no growth on FPA. Both strains were subjected to UV light mutagenesis and assayed for growth under the opposite conditions. Out of 10,000 colonies screened for each transformant, we have identified 2 mutant strains in the FRQ-null background, and 5 mutant strains in the FRQ+ background that yield the desired growth on the selective media. The circadian phenotypes of these mutations will be discussed. 1. Stuart WD, Koo K, Vollmer SJ (1988) Cloning of *mtr*, an amino acid transport gene of *Neurospora crassa*. Genome 30: 198-203.

303 **Testing a potential implication of the alternative oxidase (AOX) in the control of longevity in *Podospora anserina*** Severine Lorin, Eric Dufour, Odile Begel, Jocelyne Boulay and Annie Sainsard-Chanet. Centre de Genetique Moleculaire du CNRS, 91198 Gif-sur-Yvette, France.

Podospora anserina is an organism which presents a limited vegetative growth and a senescence phenomenon. Recently, a causal link between this senescence and respiration was demonstrated. In fact, in mutants deficient in complex IV of the respiratory chain, the longevity is strongly increased. Those mutants use exclusively an alternative respiratory pathway implicating the alternative oxidase protein (AOX), present in the inner mitochondrial membrane and accepting electrons from the quinone pool. Nowadays, several lines of evidence implicate ROS in the pathogenesis of various degenerative diseases and in ageing. On the other hand, it was recently proposed that the alternative pathway present in plants may serve to lower ROS mitochondrial production. In order to understand the potential implication of ROS and the alternative oxidase in ageing, we have tested the effects of overexpression and disruption of the alternative oxidase gene in our organism. We showed that, in mutants overexpressing the alternative oxidase gene (50 % SHAM sensitive) and in mutants disrupted for the alternative oxidase gene, longevity is unchanged. Moreover, the level of ROS seems to be the same as in the wild-type strain. Last of all, the events of mitochondrial DNA instability correlated with senescence are the same as in the wild-type strain. Taken together, our results suggest that the alternative oxidase of *Podospora anserina* cannot lower ROS production and that it has no effect *per se* on longevity. Finally, recent efforts have been directed to test the effects of overexpression of the alternative oxidase gene in mutants deficient in complex IV; preliminary results indicate that the level of global respiration may be implicated in senescence.

304 **Identification and characterisation of a histidine kinase gene in *Botrytis cinerea.*** [1]W. Cui; [2]R. E. Beever; [2]S. L. Parkes; [1]M. D. Templeton. [1]Plant Health and Development Group, HortResearch, Private Bag 92 169, Auckland, New Zealand. [2]Landcare Research, Private Bag 92 170, Auckland, New Zealand

Dicarboximide fungicides play an important role in the control of grey mould of plants caused by *Botrytis cinerea*. The appearance of fungicide resistant strains is an increasingly important consideration in the control of this fungal pathogen by chemical means. The mode of action of dicarboximide fungicides and the mechanism of their resistance have been under investigation for many years, yet remain unclear. This project aims to develop a detailed understanding of fungicide resistance in *B. cinerea* at the molecular level. A number of recent studies on laboratory mutants in 'model' fungal systems suggest that there is a correlation between dicarboximide resistance, osmotic sensitivity, and certain protein kinases. We have isolated a wide range of field strains and laboratory mutants, with low to high levels of dicarboximide resistance from *B. cinerea*. The mutants resistant to dicarboximides are mostly also osmotically sensitive. Genetic studies of *B. cinerea* have shown that resistance is conferred by a single chromosomal gene designated *Daf1* (**d**icarboximide and **a**romatic hydrocarbon **f**ungicide resistant). *Os-1* mutants of *Neurospora crassa* behave in a similar way to *Daf1* mutants of *B. cinerea*, in that they confer osmotic sensitivity and resistance to dicarboximide fungicides. We hypothesise that *Daf1* in *B. cinerea* is a homologue of the *Os-1* in *N. crassa*. We have cloned and sequenced the homologue from *B. cinerea* from both sensitive wild type strain and resistant mutant strains. Work is in the progress to clarify its relationship to *Daf1*.

305 **Hideaway, a repeated element from *Ascobolus immersus* is rDNA associated and may resemble a class I transposon.** Frank Kempken. Ruhr-Universitat Bochum, Allg & Molekulare Botanik, Bochum, Germany

In this study a genetically unstable strain from *Ascobolus immersus* stock 50 was monitored for the presence of repeated DNA. Several lambda-clones were identified from a differential hybridization approach. Among these, one lambda clone contained a repeated DNA sequence with unusual characteristics. Southern hybridization, PCR analysis and sequence analysis suggest this DNA element to be associated with rDNA sequences. Its methylation pattern is also reminiscent of rDNA. The element possesses structural characteristics of class I retrotransposons. The

data presented are discussed with respect to the ability of repeated DNA sequences to escape host defense mechanisms.

Please see also, Abstract #501

306 **Gene-tagging of *Magnaporthe grisea* growth-retarded mutants: Grm8 encodes a cross-pathway control protein.** Sheng-Cheng Wu, Zhiying Zhao, Alan G Darvill, Peter Albersheim. University of Georgia, Complex Carbohydrate Research Ctr. Athens, Georgia

Growth-retarded mutants (GRM) were screened from *Magnaporthe grisea* protoplasts mutagenized by random plasmid insertions. The grm phenotype of one mutant, GRM8, co-segregates with a selection marker on the plasmid. The tagged gene, *Grm8*, is structurally similar to those encoding cross-pathway control proteins (CPC) from other fungi. *Grm8* complements *cpc-1* mutant of *Neurospora crassa*, and thus, is a functional ortholog of *CPC-1*. The expression of *Grm8* is up-regulated during amino acid starvation, so are genes encoding, respectively, aspartate aminotransferase (*Aat1*) and carbamoyl phosphate synthase (*ARG2*). In strain C706, a *grm8* knockout mutant, expression of *Aat1* and *ARG2* is no longer enhanced by amino acid starvation. In amino acid-deficient media, C706 expectedly grows poorly as compared to its wild-type parent. However, in amino acid-rich media, C706 unexpectedly grows at a much slower rate than its parent before accelerating after 5 days, an indication that *Grm8* plays additional roles regulating *M. grisea* growth. In consistence with its phenotype in culture media, the *grm8* mutants exhibit significant reduction in pathogenicity towards rice host. *(This work was supported by U. S. Department of Energy grant DE-FG05-93ER20114 and the DOE-funded (DE-FG05-93ER20097) Center* for Plant and Microbial Complex Carbohydrates.)

307 **Influence of ambient pH and different stress conditions on Antifungal Protein expression in *Aspergillus giganteus.*** V.Meyer, M.Wedde and U. Stahl. Department of Microbiology and Genetics, Berlin University of Technology, 13355 Berlin, Germany

The Antifungal Protein (AFP) secreted by the mould *Aspergillus giganteus* is a small highly basic polypeptide of 51 amino acids. Interestingly AFP inhibits the growth of a number of filamentous ascomycetes, whereas growth of bacteria, yeasts and mammalian cells is not affected. Regulation of *afp* expression is as yet unclear but it is supposed that *afp* is regulated at the transcriptional level. In order to test conditions involved in transcriptional regulation, a reporter system (*afp::uidA*) was established. As a result it could be shown that *afp* expression is induced by alkaline ambient pH. The pH regulation of gene expression in *Aspergillus* is mediated by the transcriptional factor PacC through binding at the core consensus sequence GCCARG (Tilburn et al., 1995). To verify a PacC dependent regulation of *afp* expression, the interaction between a PacC fusion protein (Espeso et al. 1997) and the *afp* promoter was determined by electrophoretic mobility shift assays. We were able to show that the PacC fusion protein binds to two core consensus sequences within the *afp* promoter. Binding to a third putative site which exhibits one point mutation (GCCAAC) was not observed. Moreover, we investigated *afp::uidA* expression in response to different stress conditions: heat shock, osmotic stress, oxidative stress, and ethanol. All conditions tested were able to induce *afp::uidA* expression, however heat shock exposure was the strongest inducer. This result could be confirmed for AFP, since the amount of secreted AFP was significantly higher after heat shock treatment.

Tilburn et al. (1995) EMBO J. 14: 779-790. Espeso et al. (1997) J.Mol Biol. 274:466-80

308 **Characterization of the DNA binding of SRE, a GATA factor involved in iron transport in *Neurospora crassa.*** Kelly A. Harrison, George Marzluf. The Ohio State University, Biochemistry Columbus, Ohio

Several homologous genes encoding proteins involved in regulating siderophore synthesis in fungi have been isolated, including *sre* of *Neurospora*. SRE is a member of the GATA factor family, which is comprised of transcription factors that contain either one or two zinc finger motifs that recognize and bind to 'GATA' containing DNA sequences. The DNA binding properties of SRE have been characterized by electrophoretic mobility shift assays (EMSA). Results demonstrate that SRE binds specifically to DNA containing GATA sequences. EMSAs with SRE zinc finger mutants show that both zinc fingers of SRE are involved in DNA binding. EMSA results illustrate that SRE binds to a DNA probe comprised of the iron response element from the *sid1* promoter from

Ustilago. These results also show that SRE binds to the *sid1* promoter with higher affinity than to the *asd4* promoter. In contrast, NIT2, another GATA factor in *Neurospora*, binds to both *sid1* and *asd4* promoters with similar affinities. The major difference between the *sid1* and *asd4* promoters is the spacing between the two GATA sequences (25 and 10 bps, respectively). Additional experiments revealed that SRE loses its ability to bind to DNA following EDTA treatment. DNA binding by SRE, however, was rescued by adding back cadmium or zinc after EDTA treatment, therefore demonstrating that DNA binding by SRE is zinc dependent. The results taken together suggest that SRE is a two zinc finger GATA factor specific for regulating siderophore synthesis at the *sid1* promoter in *Neurospora crassa*.

309 **Identification of targets of a *b*-mediated regulation cascade in *Ustilago maydis.*** Gerhard Weinzierl[1], Andreas Brachmann[2], Regine Kahmann[1] and Joerg Kaemper[1], [1]Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch- Str., D-35043 Marburg, [2]Institute of Genetics and Microbiology, Ludwig Maximilians University, Maria-Ward-Str. 1a, D-80638 München, Germany

Pathogenic and sexual development of the fungus *Ustilago maydis*, the causal agent of smut disease on corn, is controlled by the multiallelic *b*- mating type locus encoding the two unrelated homeodomain proteins bW and bE. Upon fusion of haploid sporidia, the bE and bW proteins can form a heterodimeric complex that functions as a transcriptional regulator. In addition to genes that were shown to be directly regulated by the bE/bW complex, several genes have been isolated that are indirectly regulated via a b-dependent regulatory cascade, subjected to either positve or negative regulation. However, when deleted individually, none of these genes provided to be crucial for pathogenicity. In a genetic screen aimed to identify components of the b-dependent regulatory cascade, we have used three different b-dependently expressed genes simultaneously as reporters. Since components of the cascade could affect subsequent differentiation processes and pathogenicity by acting either as repressors or activators, we have employed both UV mutagenesis and the screening of transformants with a cDNA expression library. We will present data on the characterization of two of the UV mutants that not only show an altered expression of the reporter genes but are also affected in the expression of other b-dependent genes. A similar phenotype was observed after overexpression of the *drf1* gene that was identified via the cDNA expression library. Drf1 expression leads to the expression of various b-repressed genes, suggesting a role as transcriptional activator. We will discuss these results with respect to our current model of b-dependent gene regulation.

310 **Cloning of *pal* genes from *Neurospora crassa.*** Sergio R. Nozawa[1], Karina C. Nogueira[1], Andre Justino[1], Monica S. Ferreira-Nozawa[2], Nilce M. Martinez-Rossi[2] and Antonio Rossi[3]. [1]Departamento de Química, [2]Departamento de Genética, [3]Departamento de Bioquímica e Imunologia. Ribeirão Preto, São Paulo University, Brazil.

Although ambient pH control of enzyme secretion was first described in *N. crassa* by Nahas *et al.* (J. Gen. Microbiol. 1982, 128: 2017), the molecular responses to external pH changes are still poorly understood. In *A. nidulans*, the *pal*A, B, C, F, H and I genes are putative members of a signaling cascade involved in ambient pH sensing and the consecutive activation of genes required for growth under alkaline conditions. Thus, the molecular characterization of *N. crassa* genes homologous to *pal* genes from other fungi will help us to better understand their role in the ambient pH response. In this report, we describe the cloning and characterization of the *pal*A and *pal*F genes from *N. crassa*. *RIM20* (*pal*A homologue) and *RIM8* (*pal*F homologue) of *Candida albicans* were used to probe *N. crassa genomic* DNA digested with *EcoRI*, *BamHI* or *HindIII*. Southern-blotting bands were subcloned in the pUC18 vector and amplified in DH5alpha *E. coli* cells and the DNA fragment recovered was sequenced. We also provided evidence that both the *pal*A and *pal* F homologues from *N. crassa* genes are possibly involved in post-transcriptional and/or post-translational modification of the Pi-repressible phosphatases, controlling their secretion into the extracellular medium. Financial support: FAPESP, CNPq, FAEPA and CAPES.

311 **The lysine biosynthetic gene *lysA* of *Aspergillus nidulans* is transcriptionally regulated by the cross-pathway control of amino acid biosynthesis.** S. Busch, H.B. Bode, I. Nörenberg and G.H. Braus. Institut für Mikrobiologie und Genetik, Georg-August Universität Göttingen, Grisebachstraße 8, 37077 Göttingen

In fungi, lysine is synthesized via alpha-aminoadipate.This intermediate serves as starting material for penicillin production in some filamentous fungi like *A. nidulans* and *P. chrysogenum*. Lysine biosynthesis thus constitutes a connection between primary and secondary metabolism in *A. nidulans*. Therefore, regulation of the lysine

biosynthesis is of major interest. We investigated lysine biosynthesis in respect to a system of broad amino acid regulation. This cross-pathway control is a global regulatory network which coordinately increases transcription of several genes involved in different amino acid biosynthetic pathways upon amino acid starvation conditions or imbalances. For our studies we analyzed two *A. nidulans* lysine biosynthetic genes. The *lysF* gene, encoding homoaconitase, has been isolated and characterized by Weidner et al., 1997 and *lysA* was identified in the Oklahoma EST sequencing project and shows high amino acid sequence identities to saccharopin dehydrogenases. We isolated transcripts from liquid *A. nidulans* cultures in minimal medium with or without 3AT-induced amino acid starvation conditions. In Northern hybridization experiments we show that whereas *lysF* transcription is not affected in starved cultures, the level of *lysA* transcripts is increased about threefold. A genomic DNA fragment containing the 5' region of *lysA* revealed several putative cross-pathway regulatory elements (CPREs). This indicates that the *lysA* gene is regulated by the cross-pathway control. Investigations whether the partial regulation of lysine biosynthesis does have any influence on penicillin production are underway.

312 **Regulation of *hisHF* transcription of *A. nidulans* by adenine and amino acid limitation.** Oliver Valerius, Oliver Draht and Gerhard H. Braus. Institute of Microbiology & Genetics, Georg-August University, Grisebachstrasse 8, D-37077 Göttingen, Germany

The *hisHF* gene of *Aspergillus nidulans* encodes imidazole-glycerole-phosphate (IGP) synthase consisting of a glutamine amidotransferase and a cyclase domain. The enzyme catalyzes the fifth and sixth step of histidine biosynthesis which results in an intermediate of the amino acid and an additional intermediate of purine biosynthesis. An *A. nidulans hisHF* cDNA complemented a *Saccharomyces cerevisiae his7D* strain as well as *Escherichia coli hisH* and *hisF* mutant strains. The genomic DNA encoding the *hisHF* gene was cloned and its sequence revealed two introns within the 1659 bp long open reading frame. The transcription of the *hisHF* gene of *A. nidulans* is activated upon amino acid starvation suggesting that *hisHF* is a target gene of *cross pathway control*. Adenine but not histidine, both end products of the biosynthetic pathways connected by the IGP-synthase, represses *hisHF* transcription. In contrast to other organisms HISHF overproduction did not result in any developmental phenotype of the fungus in hyphal growth or the asexual life cycle. *hisHF* overexpression caused a significantly reduced osmotic tolerance and the inability to undergo the sexual life cycle leading to acleistothecial colonies.

313 **Comparison of additional fungal Ste20 homologues.** Michael H Perlin [1], Brisa Ramos[2], Arturo Perez Eslava[2], and Jose Maria Diaz Minguez[2]. [1]University of Louisville, Louisville, KY, USA, [2]Universidad de Salamanca, Salamanca, Spain

Signal transduction pathways are important for a variety of features of fungal development. For example, many human fungal pathogens exhibit some form of dimorphism and a common element in these and other fungi is a conserved mitogen-activated protein kinase (MAPK) pathway. This MAPK pathway plays an important role in a multitude of processes for the saprophytic fungi (e.g., *Saccharomyces cerevisiae*and *Schizosaccharomyces pombe*), in traditional human pathogens, such as *Candida albicans* and *Cryptococcus neoformans*, and in plant pathogens, such as *Ustilago maydis*. Elimination of one of the components of the three-kinase MAPK cascade was recently shown to reduce pathogenicity of *Fusarium oxysporum*. Ste20 is a protein which, among its many roles in controlling fungal development, is known to regulate the three-kinase cascade in *S. cerevisiae* and *S. pombe*. We wanted to compare the roles of Ste20 homologues in a pathogenic fungus that is not dimorphic (*F. oxysporum*) with a dimorphic fungus, that is not pathogenic (*Mucor circinelloides*). Thus, degenerate primers were used to amplify the respective regions of the Ste20 genes from both fungi. The amplified fragments showed 70-80% similarity at the predicted amino acid level. Both predicted proteins also showed up to 88% similarity with the corresponding Ste20 proteins of *S. cerevisiae* and *S. pombe*. Neither gene fragment appeared to contain any introns. The full-length copy of the Ste20 gene from *F. oxysporum* was obtained after screening a genomic library. We are designing constructs for gene disruption experiments, so as to characterize the role of these genes

314 **Allelic recombination is unaffected by gross sequence heterology between *cog* and *his-3***. P. Jane Yeadon, J. Paul Rasmussen, Frederick J. Bowring and David E. A. Catcheside. School of Biological Sciences, Flinders University, Adelaide, Australia.

Recombination between *his-3* alleles is strongly dependent on the activity of the recombination hotspot cog^L located about three kilobases 3' of this locus. Events initiated by cog^L must pass the intervening sequences in some way in

order to reach *his-3* and generate recombinant progeny. We have constructed stocks each of which includes a different DNA sequence inserted between *his-3* and cog^L. The three insertions have different lengths and no homology to each other or to the native sequence they replace. Analysis of crosses heteroallelic *his-3* K26/K480, homozygous cog^L *rec-2* and either homozygous or heterozygous for the inserted constructs has shown that lack of sequence homology over several kilobases has no significant effect on the frequency of his^+ recombinant progeny. Heterozygosity for length in this region yields a recombination frequency like that of a cross homozygous for the shorter interval, clearly showing that recombination initiated by cog^L tolerates high levels of sequence variation.

315 **Comparative studies of sequences expressed in the liquid cultured mycelia and fruiting body of *Pleurotus ostreatus.*** Seung-Ho Lee[1], Beom-Gi Kim, Kang-Hyo Lee, Gyu-Hyun Kim, Chang- Soo Lee[1], Young-Bok Yoo. Division of applied microbiology, NIAST, RDA, Suweon 441-707, Korea [1]Department of Biochemistry, Kon Kuk University, Chungju 380-701, Korea

To characterize genes involved in fruiting body development, two complementary DNA (cDNA) libraries were constructed from liquid cultured mycelia and fruiting body of *P. ostreatus*. Using single-pass sequencing from liquid cultured mycelia and fruiting body cDNA clones, respectively 948 and 629 expressed sequence taqs (ESTs) were generated. A BLASTX search revealed that 400 ESTs (42%) of liquid cultured mycelia and 344 ESTs (55%) of fruiting body showed significant similarity to protein sequences described in the NR database (E values < $1X10^{-5}$). 237 ESTs of liquid cultured mycelia (25%) and 225 ESTs of fruiting body (36%) were showed highly significant in the similarity search (E values < $1X10^{-20}$). Among total 1577 ESTs, 1075 ESTs were unigenes. When mycelia and fruiting body ESTs were compared by Blastn algorithm, 158 ESTs (10%) showed the common expression in both stages. The functional catalogs of the EST were made by comparison with functionally identified Saccharomyces cerevisiae genes. We compared ESTs of liquid cultured mycelia with fruiting body and described changes of expressed genes during fruiting body development.

316 **Differentially expressed genes in ectomycorrhiza and *Tricholoma* host specificity**. Katrin Krause[1], Angela Mankel[2], Doreen Mueller[1], Erika Kothe[1]. [1]Friedrich-Schiller-University, Dept. Microbiology, Jena, Germany [2]Philipps-University, Dept. Genetics, Marburg, Germany

From an RNA fingerprinting approach using fully developed ectomycorrhiza between *Tricholoma vaccinum* and *Picea abies vs.* pure cultures of the fungus and tree roots, more than 100 PCR fragments were identified that showed differential expression in mycorrhiza. These fragments were verified and from the 63 positive clones origin and expression pattern were checked. Of the 20 fungal genes with mycorrhiza-specific expression, sequence analyses were performed in order to identify the nature of the encoded protein *in silico*. Among them were reverse transcriptase indicating a retrotransposon in *Tricholoma*, a specifically induced aldehyde dehydrogenase pointing to plant pathogen response, and glucosidases. A gene encoding a hydrophobin specifically regulated during mycorrhization was identified and analyzed. The hydrophobin protein was detected using heterologous antiserum and protein accumulation could be shown in fungal cell walls in the hyphal mantle as well as in the Hartig net. A control using symbiotic tissue of an interaction between the fungus and a non-native host showed no hydrophobin accumulation in the Hartig net which is interpreted to show lack of regulatory functions in the non-native situation and therefore linking expression to host specificity.

317 **UPR specific transcriptional regulation in *Aspergillus niger*.** C. M. J. Sagt[1], C. A. M. J. J. van den Hondel[1,2] and P. J. Punt[2]. [1]Institute of Molecular Plant Sciences, Clusius Laboratory Leiden University, 2333 AL Leiden, The Netherlands. [2]TNO Nutrition and Food Research Institute, Department of Applied Microbiology and Gene Technology, 3700 AJ Zeist, The Netherlands

Fungi have an enormous protein secretion capacity. Homologous proteins are secreted in the g/l range, the secretion levels for heterologous proteins are 100-1000 fold less. From previous studies in S. cerevisiae it became evident that the stress caused by accumulation of proteins in the secretion-pathway, known as the Unfolded Protein Respone (UPR) serves as the major stress pathway, involved in adjusting the cell to produce heterologous proteins. This indicates that this pathway could be an important target to optimize the secretion capacity for heterolous proteins in Aspergillus niger. Tunicamycin and DTT as well as the production of some heterologous proteins are conditions were genes like BiP and CypB are upregulated. Results indicate that tunicamycin induces UPR to a level which is physiological relevant. DTT, which under the conditions used also impaires growth is a less specific (more

promiscuous) stressor. To identify unknown genes which are differentially expressed during UPR we developed a DNA macroarray technology. An A. niger cDNA library is spotted on high density filters (4608 clones) and will be used for hybridisation with radiolabelled cDNA. The redundancy of this library, from which more than 95% of the clones contain an insert, was determined by probing the filters with labelled DNA fragments from highly expressed genes BiP and GPD. The cDNA probes used for hybridisation of the macro-arrays were obtained from Aspergillus niger wild-type and strains stressed with DTT or tunicamycin. In addition, RNA was also isolated from single chain antibody fragment producing Aspergillus niger which was shown to give an UPR response. After RNA isolation, reverse transcription in the presence of [33P]dCTP, hybridisation and normalization of the signals using the beta-lactamase and or gpdA gene, differentially expressed clones were identified. Several clones with a differential transcriptional behaviour will be analysed in further detail.

318 **Circadian regulation of the light input pathway in *Neurospora crassa*.** Martha Merrow[1], Lisa Franchi,[2, 3] Zdravko Dragovic[1], Margit Görl[4], Judy Johnson[5], Michael Brunner[4], Giuseppe Macino[3], and Till Roenneberg[1]. [1] Institute for Medical Psychology, University of Munich 80336 Munich, Germany. [2] Department of Biology New York University, New York, NY, USA. [3] Dipartimento di Biotecnologie Cellulari Ed Ematologia, Università di Roma 00185 Rome, Italy. [4] Institute for Physiolgical Chemistry University of Munich 80336 Munich, Germany. [5] Institute for Immunology, University of Munich, 80336 Munich, Germany

FREQUENCY (FRQ) is a critical element of the circadian clock of *Neurospora crassa*. The *white collar* genes are important both for light reception and clock function. We show that FRQ interacts genetically and physically with WHITE COLLAR 1 (WC-1), and physically with WC-2. As a consequence of these interactions, the responsiveness of the light input pathway is clock controlled. This circadian modulation extends to light- inducible components and functions which are not rhythmic themselves in constant conditions. These findings begin to address how clock components interact with basic cellular functions, in this case with sensory transduction.

319 **Gene inactivation in the ascomycete. *Podospora anserina*** Andrea Hamann, Heinz. D. Osiewacz. Botanisches Institut, J. W. Goethe-Universitat, Marie-Curie-Str. 9, D-60439 Frankfurt, Germany

Podospora anserina is a filamentous ascomycete closely related to *Neurospora crassa*. In *Neurospora*, a specific gene inactivation mechanism, termed RIP (repeat-induced point mutation), was identified in the late eighties. It results in the irreversible inactivation of duplicated sequences. Subsequently, it has repeatedly been suggested that RIP may have a broader distribution in fungi. Our recent identification of two highly degenerated transposons in *P. anserina* suggested that RIP may have led to the observed specific transposon mutations. We analyzed a transformant strain of *P. anserina* carrying a duplication of the *Grisea* gene encoding the copper-regulated transcription factor GRISEA. One of the offspring of a cross between this transformant strain and the wild type displayed the grisea mutant phenotype although the strain contains a complete ectopically integrated *Grisea* gene. Sequencing of the cDNA derived from this gene revealed three mutations in the coding sequence. Most likely, these mutations are the reason for the loss-of-function phenotype. The type of mutation and the fact that it was demonstrated in a strain that went through meiosis clearly demonstrates that a mechanism similar to RIP in *N. crassa* is operating also in *P. anserina*. Furthermore, it is well possible that this type of mechanism is more widespread and may have a more general impact on protecting strains against deleterious processes as they may be the result of transposon mobility or the recombination between duplicated sequences.

320 **Nutritional profiling technology for high-throughput functional genomics analysis of filamentous fungi.** Matthew M. Tanzer, Amy R. Skalchunes, Ryan W. Heiniger, Blaise A. Darveaux, Herbert N. Arst, Jr.*, and Jeffrey R. Shuster. Department of Microbial Phenomics, Paradigm Genetics, Inc. 108 Alexander Dr. RTP, NC. *Imperial College School of Medicine. Department of Infectious Diseases. Du Cane Rd. London

Nutritional profiling is a high-throughput technology for the analysis of fungal growth on numerous carbon, nitrogen, and metabolites, in an automated fashion. By examining the changes in nutrient utilization, inferences can be made about the pathways and processes that are affected by a particular gene or chemical treatment. Two example studies are presented here. First, the relationship of 21 Aspergillus nidulans areA mutant lines was studied for their utilization of 95 nitrogen sources. areA encodes a transcriptional activator mediating nitrogen metabolite repression. Hierarchical clustering of strains based on the overall utilization patterns of these nitrogen sources was performed for these strains. Data will be presented showing the relationships among all strains and allele pair

clustering. These data are largely in agreement with the published phenotypic data that was based on a limited set of nitrogen sources. Second, the relationship of 95 carbon sources was examined with respect to carbon catabolite repression. Wild-type A. nidulans strains were grown in these plates with various concentrations of allyl-alcohol. Allyl alcohol is converted to acrolein by the action of alcohol dehydrogenase (adh). A. nidulans will grow in the presence of allyl alcohol on repressing carbon sources but will be sensitive on non-repressing carbon sources where adh is expressed. Hierarchical clustering of the carbon sources based on the level of utilization/repression in the presence of allyl alcohol will be presented. Nutritional profiling is a powerful system to rapidly analyze the nutritional requirements of filamentous fungi by including far more nutrient sources than have traditionally been used in standard laboratory experiments. This information will promote the understanding of gene function or chemical action.

321 **Histone deacetylases in *Aspergillus nidulans*.** Stefan Graessle, Peter Loidl, Markus Dangl, Patrick Trojer, Hubertus Haas, Karin Mair, and Gerald Brosch. Department of Microbiology, Medical School, University of Innsbruck, A 6020 Innsbruck, Austria.

In eukaryotes, DNA and core histones constitute the nucleosome, which is the essential structural unit for packing eukaryotic DNA into chromosomes. In addition to their structural function, nucleosomes are thought to play a role in the regulation of transcription. Core histones are susceptible to a wide range of posttranslational modifications, including phosphorylation, methylation, and acetylation. Hyperacetylation preferentially occurs in actively transcribed chromatin regions because the neutralization of the positive charge by acetylation of the epsilon-amino group of specific lysine residues within the N-terminal domain of core histones has been proposed to lead to loosening histone-DNA contacts, thereby facilitating the accessibility of various transcription factors to DNA. Core histones can be acetylated by histone acetyltransferases (HATs), such as the yeast transcription factor GCN5, the transcriptional adapter p300/CBP or its associated factor P/CAF. This acetylation process is reversed by a second class of enzymes, histone deacetylases (HDACs) which form highly conserved protein families in many eukaryotic species. Using different PCR approaches we have identified and characterized at least five new members of different HDAC families in *Aspergillus nidulans*. Expression studies showed specific transcripts and indicated different expression levels of the genes. Expression of some of the genes was induced when cells were treated with HDAC inhibitors like Trichostatin A. Recombinant HDACs expressed in prokaryotic expression systems were measured for enzyme activity and purified recombinant proteins were used to produce polyclonal antibodies for the identification of active enzyme fractions of the fungus.

322 **SREA deficiency leads to derepression of siderophore transport, intracellular siderophore accumulation and oxidative stress in *Aspergillus nidulans.*** Hubertus Haas, Harald Oberegger, Michelle Schoeser, Beate Abt, and Ivo Zadra. Department of Microbiology, University of Innsbruck (Med. School), Innsbruck, Austria

Under conditions of iron deficiency most fungi excrete siderophores - low molecular-mass ferric iron chelators - in order to mobilize extracellular iron. In *A. nidulans*, siderophore biosynthesis has been shown to be negatively regulated by the GATA-type transcription factor SREA. Furthermore, lack of SREA leads to derepression of siderophore uptake and metabolization (e.g. siderophore hydrolysis by ornithine esterase) as well as increased accumulation of ferricrocin, the siderophore responsible for intracellular iron storage. In *sreA*-deletion strains, derepression of extracellular siderophore production is only partial, indicating the presence of additional iron regulatory mechanisms. In contrast to siderophore excretion, ferricrocin accumulation is positively affected by the external iron availability suggesting a protective role of this siderophore in detoxification of intracellular iron excess. The harmfulness of deregulated iron uptake in this mutant is demonstrated by increased expression of genes encoding the antioxidative enzymes superoxide dismutases SODA and SODB and catalase CATB. Noteworthy, expression of *catB* was found to be repressed by iron starvation in wild type and sreA-mutant strains consistent with catB being subject to positive iron regulation. Differential display led to identification of the putative SREA target genes *amc-1* and *mirA*. Since *amc-1* encodes a puative mitochondrial carrier for the siderophore component ornithine, cross regulation of siderophore biosynthesis and ornithine metabolism is indicated. The deduced MIRA amino acid sequence displays significant similarity to recently characterized siderophore permeases of Saccharomyces cerevisiae. Northern analysis confirmed iron-dependent expression of *amc-1* and *mirA* and the role of SREA as a repressor of gene expression. These data demonstrate that SREA regulates a variety of processes involved in siderophore metabolism and show that increased iron uptake leads to oxidative stress in *Aspergillus*. This work was supported by Austrian Science Foundation Grant FWF-P13202-MOB.

323 **Using transcriptional profiling in *Neurospora crassa* to identify circadian clock-controlled genes and mediators of clock-regulated conidiation.** Alejandro Correa[1], Zachary Lewis[1], Xie Xin[2], Daniel Ebbole[2] and Deborah Bell-Pedersen[1]. [1]Department of Biology and [2]Department of Plant Pathology, Texas A&M University, College Station, TX, 77843.

In *Neurospora crassa*, the circadian clock provides an endogenous signal to regulate the timing of asexual spore development (conidiation). In constant darkness, conidiation occurs once every 22h during the subjective morning. Several environmental signals can also initiate conidiation in *N. crassa*, including light, air, carbon and nitrogen starvation. Three key regulatory loci of the conidiation pathway are known and include acon-2, fl and acon-3. Different combinations of the regulators are thought to control the expression of downstream conidiation specific genes (con-10, con-6 and eas (ccg-2)). Our recent results indicate that the clock impinges early in the developmental pathway to regulate production of aerial hyphae and that clock regulation of eas (ccg-2) requires a functional acon-2 or fl gene, but not both. We are currently using N. crassa cDNA microarrays to help us understand the mechanisms by which the clock regulates this and others critical cellular events. Sets of probes obtained from different times of the day are allowing us to catalog rhythmically expressed genes. We predict that the initial clock signal for aerial hyphae production will be expressed early after development is induced and will be rhythmically expressed. In attempts to identify this signaling factor(s), we are probing the arrays with labeled cDNA produced from mRNA isolated from cultures harvested early after developmental induction. Putative candidate cDNA clones that are rhythmically expressed and activated early during development are being sequenced in their entirety. Constructing null alleles will test the role of these genes in the circadian clock and in development.

324 **Analysis of the *bzuA* gene encoding the cytochrome P450 benzoate para-hydroxylase in *Aspergillus nidulans.*** James A. Fraser, Meryl A. Davis and Michael J. Hynes. Department of Genetics, University of Melbourne, Australia

Amide utilisation by *A. nidulans* has been extensively studied in our laboratory through the cloning and analysis of regulation of the amidase encoding genes *amdS*, *fmdS* and *gmdA*. Two unlinked loci have previously been identified as being required for the utilisation of benzamide as the sole nitrogen source. The *gmdA1* mutation defines the general amidase structural gene required for hydrolysis of long chain and some aromatic amides (including benzamide), whilst the *bzuA1* mutation defines an unknown function.

We have cloned the *bzuA* gene via a chromosome walk on chromosome IV and found that it encodes a protein belonging to the cytochrome P450 superfamily, and is orthologous the benzoate para-hydroxylase (*bphA*) in *Aspergillus niger*. The *bzuA1* phenotype was shown to be due to an intracellular accumulation of benzoate following benzamide hydrolysis by GmdA, resulting in growth inhibition. Benzoate serves as a sole carbon source and multiple binding sites for the *A. nidulans* CreA protein responsible for carbon catabolite repression have been identified in the *bzuA* promoter, resulting in expression responding to carbon limitation. As similarity between BzuA and BphA is lowest at the N-terminal (the region required in cytochromes P450 for anchoring in the endoplasmic reticulum), a BzuA:GFP fusion was generated. Fluorescence microscopy revealed fusion of GFP to the first third of the BzuA protein was sufficient to generate a reticulate fluorescence pattern, indicative of BzuA being localised to this organelle.

325 **Structural and functional analysis of citrate synthase gene citA in *Aspergillus nidulans.*** Soon Won Seo[1], Ji Young Bang[1], Keon Ho Han[1], Cheong Ho Lee[2], Jeong Goo Lee[1] and Pil Jae Maeng[1]. [1]Chungnam National University, Microbiology, Daejeon, Korea. [2]Korea Ginseng & Tobacco, Daejeon Korea

Citrate synthase (EC 4.1.3.7) which catalyzes the condensation reaction between acetyl-CoA and oxaloacetate yielding citrate, the first step of the TCA cycle, functions as a rate-limiting enzyme of the cycle. As a subsequent study to the previous reports on the citrate synthase (CitA; Maeng et al., 1994, Kor. J. Microbiol. 31: 586-593) and its gene (citA; Park et al., Mol. Cells 7: 290-295) isolated from *Aspergillus nidulans*, we cloned and analyzed the structure of the citA cDNA. The citA cDNA was shown to encode a protein of 52.2 kDa consisting of 474 amino acid residues. A 1.45-kb fragment containing citA promoter (citA- p) was cloned and sequenced. The promoter contains three putative CAAT motifs, two CT boxes, and three candidate sequence for CreA (catabolite repression protein in Aspergillus) binding sites. The mode of citA expression was followed by confocal microscopic and spectrofluorometric analysis of the transformants containing a citA-p::sgfp gene fusion integrated at trpC locus. On

asexual growth stage, the expression of citA was found to be highly encouraged by sodium acetate, but severely repressed by glucose. Furthermore the repression was synergistical in the presence of both glucose and glutamate. By deletion analysis of the citA-p, we found a genuine CreA-bindng motif responsible for the catabolite repression by glucose. On sexual stage, no significant expression was detectable in any of the above medium, which suggests that there exists some relationship between the regulation of citA expression and sexual or asexual differentiation of *A. nidulans*.

326 **Functional domains and binding properties of *Aspergillus nidulans* AmyR**. Shuji Tani, Yoko Katsuyama, Masashi Kato, Tetsuo Kobayashi, and Norihiro Tsukagoshi. Department of Biological Mechanisms and Functions, Graduate school of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

The amyR gene of *A. nidulans* encoded a cys-6 zinc transcriptional activator (AmyR) of 662 amino acid residues. The truncated AmyR1-411 of *A. nidulans* was produced as an MBP fusion protein in E. coli. The MalE::AmyR1-411 was purified by amylose affinity column, and used in gel mobility shift assay and DNase I footprinting. The MalE::AmyR1-411 bound to both the CGGN8CGG sequence of the a- glucosidase A promoter and the CGGAAATT sequence of the Taka-amylase A promoter, as described for *A. oryzae* AmyR. The MalE::AmyR1-411 had almost the same affinity to both sequences. Mutation in both or either of the CGG triplets in CGGN8CGG resulted in a drastic decrease in the binding affinity to MalE::AmyR1-411. The mutational analysis within and around the CGGAAATT sequence of the Taka-amylase A promoter revealed that MalE::AmyR1-411 required not only the CGG triplet but also the following AAATT sequence for its binding. AmyR possessed five homologous domains (Zn and MH1-MH4) to Mal63p, a transcriptional activator for the genes involved in maltose utilization in *Saccharomyces cerevisiae*. An AmyR derivative, AmyRZn2, lacking the MH3 and MH4 domains functioned as a constitutive activator in *A. nidulans*, suggesting that MH3 and/or MH4 may be the negative regulatory domain. AmyRZn1 lacking MH2-4 could not complement the growth defects of the amyR disruptant, and thus MH2 is indispensable for the transactivation. Interaction between the activation domain and the negative regulatory domain might be modulated in response to maltose.

327 **Both a Cys2His2 zinc finger motif and a Zn(II)2Cys6 binuclear cluster motif of *Colletotrichum lagenarium* Cmr1p are indispensable for the regulation of melanin biosynthesis.** Gento Tsuji, Osamu Horino and Yasuyuki Kubo. Laboratory of Plant Pathology, Kyoto Prefectural University, Kyoto 606-8522, Japan.

Colletotrichum lagenarium is a plant pathogenic fungus that produces melanin during the appressorial differentiation stage of conidial germination and during the late stationary phase of mycelial growth. We previously reported the identification of genes for a unique transcription factor, CMR1 (Colletotrichum melanin regulation), which is involved in mycelial melanin biosynthesis. Cmr1p contains two distinct DNA- binding motifs, a Cys2His2 zinc finger motif and a Zn(II)2Cys6 binuclear cluster motif. To determine whether the two putative DNA binding motifs are functional *in vivo*, CMR1 deletion constructs were designed and transformed into the *cmr1* null mutant. The constructed plasmids were pCRC6-1, pCRZf-1, and pCRnon-1 that lack a Cys2His2 zinc finger motif, a Zn (II)2Cys6 binuclear cluster motif, and both DNA binding motif sequence, respectively. Transformants obtained with pCRC6-1 produced melanin. However the extent of melanization in the transformants was clearly lower than that in wild type. No wild type recombinants was obtained with pCRZf-1, and pCRnon-1. Thus, both these putative DNA binding motifs are necessary for wild type levels of melanization in culture.

328 **Promoter analysis of the *xynG2* gene in *Aspergillus oryzae* KBN616.** Tetsuya Kimura, Takeshi Aburatani, Hayato Suzuki, Toshiyasu Matsuoka, Kazuo Sakka and Kunio Ohmiya. Faculty of Bioresources, Mie University, Tsu Mie Japan

Aspergillus oryzae is a very important fungus in Japan for food and enzyme production. Especially, in soy source production, degradation of soybean cell wall fraction is a limiting step of efficient usage of raw materials. Hemicellulose is one of the major components of plant cell walls. Therefore, degradation of hemicellulosic compounds is one of the key steps for soy source production. *A. oryzae* produces several kinds of hemicellulosic enzymes including xylanase when grown on hemicellulose. To understand the expression mechanism of the xylanase genes of *A. oryzae* , we cloned six genes encoding xylanase from *A. oryzae* KBN616 that is used for soy source production. Family 11 xylanase XynG2 is the major xylanase when *A. oryzae* grow on the Xylan medium. Expression analysis of the promoter of *xynG2-the Escherishia coli uidA* fusion gene (GUS) in *A. oryzae* was carried

out. Promoter region of *xynG2* contains two putative XlnR binding consensus sequences, 5'GGCTAAA3'. Deletion analysis suggested that they are essential for *xynG2* expression in xylan medium. Point mutational analysis of both sites indicated that they are independently functional in vivo for the induction by xylan.

329 **A short N-terminal region adjacent to the central core region of AoHapC, a subunit of the *Aspergillus oryzae* CCAAT binding complex, is necessary for enhancement of gene expression.** Masashi Kato, Ken-ichi Kamei, Ryoko Oonishi, Shinjiro Tanoue, Tetsuo Kobayashi and Norihiro Tsukagoshi. Nagyoya University, Nagoya 464-8601, Japan.

A. nidulans Hap complex has been shown to regulate expression of several fungal genes and contain a subunit encoded by the *hapC* gene, a homologue of the *HAP3* gene of *S. cerevisiae*. Recently, we have shown that an *A. oryzae* CCAAT binding protein, AoCP, is homologous to the *A. nidulans* Hap complex. Furthermore, *AohapC*, a *hapC* homologue, was isolated and shown to be functionally interchangeable to the *A. nidulans hapC*, as judged by the activity of the *A. nidulans* Hap dependent- enzyme endo-beta-1, 4- glucanase A (EG-A encoded by the *eglA* gene). The central "core" region of AoHapC (aminoacid residue 48 to 131) was highly conserved among the HAP3 homologues and could be involved in DNA binding and subunit association. In order to characterize functional domains of AoHapC, mutant *AohapC* genes lacking an N-terminal region, a C-terminal region and both regions were constructed and introduced to an *A. nidulans hapC* deletion strain to obtain the transformants, dN, dC and dNdC, respectively. Electrophoretic mobility shift assays showed that the mutants dN and dC possessed the CCAAT binding activity, indicating that the core region of AoHapC is sufficient to the CCAAT binding activity. However, the EG-A activity of dN was as low as that of the *hapC* disruptant, while the EG-A activity of dC was almost the same as that of the wild type strain. The same results were obtained with the *taa* gene. These results indicate that the N-terminal region as well as the core region is required to enhance gene expression. Further analysis with a series of N-terminal deletions of AoHapC revealed that a short N-terminal region adjacent to the core region is involved in the transcriptional enhancement of the *eglA* gene.

330 ***Colletotrichum lagenarium CST1* (*Colletotrichum* sterile12-like), a potential downstream transcriptional factor of a MAP kinase has homeodomain and Cys2His2 zinc finger DNA binding motifs.** Yasuyuki Kubo, Gento Tsuji and Osamu Horino. Laboratory of Plant Pathology, Kyoto Prefectural University,Kyoto 606-8522, Japan.

The infection of *Colletotrichum lagenarium*, the causal agent of cucumber anthracnose disease, involves several key steps: germination; formation of melanized appressoria; appressorial penetration; and subsequent invasive growth in host plants. *C.lagenarium CMK1*encoding a mitogen-activated protein (MAP) kinase of yeast *FUS3/KSS1* homologue plays a pivotal role in these infection steps. *Saccharomyces cerevisiae* Ste12p is a transcriptional factor that plays a key role in coupling signal transduction through Fus3/Kss1 MAP kinase modules to specific gene expression required for mating and pseudohyphal filamentous growth. Also in pathogenic yeasts of *Candida albicans* and *Filobasidiela neoformans,* Ste12p homologues play similar roles during dimorphic transitions. In filamentous fungi, *Aspergillus* Ste12p homologue SteAp is required for sexual reproduction. We cloned *STE12* like *CST1* (*Colletotrichum* sterile12-like) from plant pathogenic filamentous fungi *C.lagenarium* encoding a protein with highly conserved homeodomain found in other Ste12 proteins. Cst1p also had C-terminal Cys2His2 zinc finger DNA binding domains retained in Ste12Ap of *A. nidulans* but not present in the other Ste12 proteins.

331 **Cloning, nucleotide sequence and characteristic of two mutants of the *suDpro* gene from *Aspergillus nidulans* encoding a putative glycoprotease.** A. Grzelak, E. Szewczyk, P. Borsuk, J. Empel, A. Pollak, J. Rozyczka and P. Weglenski Department of Genetics, Warsaw University, Warsaw, Poland

In *Aspergillus nidulans* arginine can serve as a source of proline in the wild-type strain and in proline mutants blocked in one of the first two steps of the main pathway of proline synthesis. The arginine regulation system is connected with a specific activator gene (*ArcA*) (poster Empel J. et al.) and several repressor genes (*suApro, suDpro, suEpro,suHpro, suJpro* and *suLpro*). Supressors of proline mutations were obtained as mutants with derepression of both arginine catabolic enzymes *agaA* coding for arginase and *otaA* coding for OTA-se (see poster Dzikowska A. et al.). The *suDpro* gene is particularly interesting from the group of supressor gene mutants. Two mutants in this gene: *suD25pro* and *suD19pro* have similar pleiotropic phenotypes. We have cloned the *suDpro* gene by complementation of the mutation in the *suD25pro* strain with the wild type gene. The cDNA clone was selected from an *A. nidulans*

cDNA library. Both the genomic and cDNA clones were sequenced. Comparison of these sequences revealed the presence of one 66 bp long intron. The open reading frame of the *suDpro* gene transcript potentially encodes a protein of 363 amino acids. The predicted amino acid sequence shows significant homology with glycoproteases of procaryotic or eucaryotic origin and contains a putative zinc-binding site. The PCR method was used to determine sequences of the *suDpro* gene in the *suD19pro* and *suD25pro* mutants. In the *suD25pro* strain there is a point mutation at the beginning of the sequence, producing a stop codon at position 101. In the *suD19pro* strain there is a deletion of 4 amino acids (including one conserved). The presumable functions of this suppressor gene are discussed. This work was supported by grant from the Polish State Committee for Scientific Research 6 P04A 00519 and partly by Department of Biology grant number BW/1485/47.

332 **Molecular characterization of *in planta* induced gene, *PIG1* , in *Magnaporthe grisea*.** Ahn, Namsook. Soeul National University. Agric. Biotechnology, Kyonggi South Korea

Magnaporthe grisea is the causal fungus of rice blast, the most devastating rice disease in the world. Although much has been learned about early infection processes by this fungus, little information is available on the late stages of infection; *in planta* growth and symptom development. A cDNA showing a high sequence homology to *Bli-3* of *Neurospora crassa*, a blue light-inducible gene with unknown function, was isolated from *M. grisea* infected rice cDNA library and designated as *PIG1* (in *P*lanta *I*nduced *G*ene). *PIG1* contains an open reading frame of 627 nucleotides which encode 208 amino acid residues. The estimated molecular weight was 22.8 KDa with pI of 6.4. Southern blot analysis of genomic DNA revealed that the *PIG1* exists as a single copy in the haploid genome of *M. grisea*. Expression of *PIG1* was detected at a low level during the growth in a complete medium, but was highly up-regulated during symptom development in rice. To evaluate the role of *PIG1* in fungal pathogenicity and morphogenesis at the molecular level, a null mutant, *pig1*, was obtained by gene knock out strategy. The phenotypic characterization of a null mutant will be presented.

333 **Transcriptional control of the gibberellin biosynthesis in *Gibberella fujikuroi.*** Martina Mihlan and Bettina Tudzynski. Westfälische Wilhelms-Universität Münster, Institut für Botanik, Schlossgarten 3, 48149 Münster, Germany

In the last years much knowledge has been gained about gibberellin (GA) biosynthesis, mainly due to the cloning of genes encoding biosynthetic enzymes in *Gibberella fujikuroi*. Recent studies have shown that at least 6 genes of the gibberellin (GA)-biosynthetic pathway are clustered in chromosome 4 in the *G. fujikuroi* genome; these genes encode the bifunctional *ent*-copalyl diphosphate synthase/*ent*-kaurene synthase (*cps/ks*), a GA-specific geranylgeranyl diphosphate synthase (*ggs2*) and four cytochrome P450 monooxygenases. (Tudzynski et al. 1998, Tudzynski and H÷÷lter,1998). The availability of the genes allows the study of mechanisms of gene regulation on a molecular level. Recently, we could show that gene replacement of the major nitrogen regulatory gene, *areA*, led to a significant reduction of gibberellin formation by repressing the expression of all but one GA-pathway genes (Tudzynski et al. 1999). Complementation of the *areA*-deficient mutant with the *areA* wild-type copy completely restored the ability to produce gibberellins as well as the high expression level of five GA genes which suggests that the positive acting regulatory protein AREA directly controls the transcription of the pathway genes. In contrast to these genes, the expression of *P450-3* is not affected by high amounts of ammonium. In order to find other putative transcription factors beside AREA which can also bind to the promoters of the GA biosynthetic genes, intact, mutated and size-reduced promoter-fragments of the genes *P450-1/P450-4* (double promoter, AREA-regulated) and *P450-3* (not influenced by AREA) were fused with the *gus* reporter gene. Putative binding sites of transcription regulators are discussed. References B. Tudzynski, H. Kawaide, and Y. Kamiya (1998). Curr. Genetics, 34: 234-240. B. Tudzynski and K. Holter (1998). Fungal Genetics and Biology, 25: 157-170. B. Tudzynski, V. Homann, B. Feng, and G.A. Marzluf (1999). MGG, 261: 106-114.

334 **Ste20/Pak1 kinase homologs regulate differentiation and virulence of *Cryptococcus neoformans*.** Wang, P.[1], Breeding, C.S.[1], Lengeler, K.B.[1], Cardenas, M.E.[1], Cox, G.M.[2,3], Perfect, J.R.[2,3], and Heitman, J.[1,2,3,4,5] Departments of Genetics[1], Medicine[2], Micro.[3], and Pharma. and Cancer Biology[4], the H. Hughes Med. Inst.[5], Duke University Medical Center, Durham, NC 27710

Cryptococcus neoformans is an opportunistic fungal pathogen with a defined sexual cycle involving mating between haploid MATalpha and MATa cells. Interestingly, virulence of this organism has been linked to mating type, and

MATalpha cells are more virulent than congenic MATa cells. We are studying signal transduction pathways that regulate mating and virulence to understand how mating type might be linked to virulence. Here we describe the identification of three genes encoding homologs of the Ste20/Pak1 kinases from *S. cerevisiae* and humans: *MATalpha*, *STE20a*, and *PAK1*. The Ste20 homologs are mating type specific; the MATalpha locus contains the *MATalpha* gene and the MATa locus contains the related but divergent *STE20a* gene. The *MATalpha*, *STE20a* and *PAK1* genes were disrupted by transformation and homologous recombination in serotype A and congenic serotype D strains of *C. neoformans.* These studies reveal the Ste20 and Pak1 kinases play roles in mating, haploid fruiting, cytokinesis, and virulence. The *pak1* mutation was synthetically lethal with either *MATalpha* or *ste20a* mutations, indicating that these related PAK kinases may share partially overlapping functional roles. In summary, our studies reveal diverse functions for the Ste20 and Pak1 kinases in differentiation and virulence, identify *MATalpha* as a component encoded by the MATalpha locus that is associated with virulence, and provide evidence that virulence of *C. neoformans* is regulated by a specialized MAP kinase signaling cascade.

335 **Molecular characterization of in planta induced gene, PIG1, in *Magnaporthe grisea*.** Namsook Ahn, Soonok Kim, and Yong-Hwan Lee. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea.

Magnaporthe grisea is the causal fungus of rice blast, the most devastating rice disease in the world. Although much has been learned about early infection processes by this fungus, little information is available on the late stages of infection; *in planta* growth and symptom development. A cDNA showing a high sequence homology to *Bli-3* of *Neurospora crassa*, a blue light-inducible gene with unknown function, was isolated from *M. grisea* infected rice cDNA library and designated as *PIG1* (in *P*lanta *I*nduced *G*ene). *PIG1* contains an open reading frame of 627 nucleotides which encode 208 amino acid residues. The estimated molecular weight was 22.8 KDa with pI of 6.4. Southern blot analysis of genomic DNA revealed that the *PIG1* exists as a single copy in the haploid genome of *M. grisea*. Expression of *PIG1* was detected at a low level during the growth in a complete medium, but was highly up-regulated during symptom development in rice. To evaluate the role of *PIG1* in fungal pathogenicity and morphogenesis at the molecular level, a null mutant, *pig1*, was obtained by gene knock out strategy. The phenotypic characterization of a null mutant will be presented.

336 ***ArcA*, the regulatory gene of the arginine catabolic pathway in *Aspergillus nidulans***. J.Empel, I.Sitkiewicz, A.Andrukiewicz, K.Lasocki, P.Borsuk and P.Weglenski. Department of Genetics, Warsaw University, Warsaw, Poland

The *arcA* gene codes for a transcriptional activator necessary for the high level expression of two genes of the arginine catabolic pathway in *Aspergillus nidulans*: the *agaA* (coding for arginase) and the *otaA* (coding for ornithine transaminase, OTAse) (see posters Grzelak et al. and Dzikowska et al.). Here we present the complete genomic and cDNA nucleotide sequences and the pattern of expression of the *arcA* gene. This gene contains one intron and encodes a polypeptide of 600 amino acids. The deduced protein belongs to Zn_2Cys_6 fungal regulatory proteins. ARCA is the first known protein of this family, which at the fifth position of the second, six amino acid, loop of the Zn cluster domain has glycine instead of the conserved proline. We have established that the transcription of the *arcA* gene is not self-regulated and does not depend on arginine. Two mutations: $arcA^{d}47$, gain-of-function and $arcA^{r}3$, loss-of-function, mapping in the *arcA* gene have been sequenced and effects of these mutations on the expression of the *agaA* gene at the transcriptional level were shown.

This work was supported by grant from the Polish State Committee for Scientific Research 6 P04A 0013 18.

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337 **The *Aspergillus nidulans* multimeric CCAAT binding complex AnCF is negatively autoregulated via its *hapB* subunit gene.** Stefan Steidl*, Michael J. Hynes** and Axel A. Brakhage*. *Institut fuer Mikrobiologie und Genetik, TU Darmstadt, Germany **Department of Genetics, University of Melbourne, Parkville, Australia

Cis-acting CCAAT elements are frequently found in eukaryotic promoter regions. Many of them are bound by conserved multimeric complexes. In the fungus *Aspergillus nidulans* the respective complex was designated AnCF

(*A. nidulans* CCAAT binding factor). AnCF is composed of at least three subunits designated HapB, HapC and HapE, with each subunit being indispensable for AnCF function***. Here, we show that the promoter regions of the *hapB* genes in both *A. nidulans* and *A. oryzae* contain two inversely oriented, conserved CCAAT boxes (box alpha and box beta). AnCF binding in vitro to these boxes occurs in a non-mutually exclusive manner. Western and Northern blot analyses showed that steady state levels of HapB protein as well as *hapB* mRNA were elevated in *hapC* and *hapE* deletion mutants. Mutagenesis of box beta led to a 5-fold reduced expression of a *hapB-lacZ* gene fusion compared with the expression derived from a wild-type *hapB-lacZ* fusion. In contrast, in a *hapB* deletion background the *hapB-lacZ* expression level was elevated. Overexpression of *hapB* using an inducible *alcA-hapB* construct strongly repressed the expression of an *hapB-lacZ* gene fusion. These results indicate that (i) box beta is an important positive *cis*-acting element in *hapB* regulation, (ii) AnCF does not represent the corresponding positive *trans*-acting factor and (iii) that AnCF is involved in repression of *hapB*.

***Steidl, S., Papagiannopoulos, P., Litzka, O., Andrianopoulos, A., Davis, M. A., Brakhage, A. A., and Hynes, M. J. 1999 Mol. Cell. Biol. 19: 99-106

338 **Functional *in vivo* analysis of *otaA* - arginine catabolism gene in *Aspergillus nidulans***. A. Dzikowska, M. Koper and P.Weglenski. Department of Genetics, Warsaw University, Warsaw, Poland

In *A. nidulans* arginine can serve as a source of proline and it can also be utilized both as a carbon and nitrogen source. Utilization of arginine as a source of proline depends on the presence and inducibility of arginine catabolic enzymes, arginase and ornithine transaminase (OTAse) which are encoded by *agaA* and *otaA* genes, respectively (1,4). The induction of both genes by arginine is subject to specific control, both positive and negative. The arginine specific regulation was shown to be exerted at the level of transcription. It was shown that there are several repressor genes (*suA, suD, suE, suH, suJ and suL*) and one activator gene (*arcA*) (5) (see also posters Empel J. et al. and Grzelak A. et al).The expression of *agaA* and *otaA* genes is also controled by systems of general carbon and nitrogen metabolite repression mediated by the *creA* (3) and *areA* (2) gene products, respectively. The promoter region of the *otaA* gene contains AREA and CREA binding sites which are protected by AREA and CREA *in vitro* (4). A system for the functional analysis of the *otaA* gene *in vivo* has been worked out. The system is based on the integration of a single copy of the *otaA* gene into the *uaZ* locus (6). Using this system and the mutational analysis of the *otaA* gene promoter we have identified a region which is involved in arginine induction. It has also been shown that the GATT sites bound by the AREA *in vitro* are probably not an AREA physiological binding sites. It is possible that they are bound by some negatively acting regulatory protein. Point mutations in the CREA binding sites result in *otaA* derepression. Similarly as a removal of the CREA binding sites or multicopy integration of the *otaA* gene, they result in the supression of the *pro*$^{-}$ mutation. References: 1. Borsuk P, Dzikowska A, Empel J, Grzelak A, Grzeskowiak R and Weglenski P(1999) Acta Biochimica Polonica 46: 391-403 2. Caddick MX, Arst, HN, Taylor LH, Johnson LI and Brownlee A (1986) EMBO J. 5:1087-90. 3. Dowzer CEA, Kelly JM (1991) Mol Cell Biol 11:5701-5709 4. Dzikowska A, Swianiewicz M, Talarczyk A, Wisniewska M, Goras M, Scazzocchio C, Weglenski P (1998) Curr Genet 35:118-126. 5. Empel J (1998) Cloning and characterisation of the *arcA* gene of *A. nidulans*. PhD Thesis. Dept. Genetics, Warsaw University 6. Oestreicher N, Sealy-Lewis H and Scazzocchio C. (1993) Gene 132:185-192

339 **Characterization of histone deacetylases in *Cochliobolus carbonum*.** Eva M. Brandtner[1], Peter Loidl[1], Markus Dangl[1], Patrick Trojer[1], Jonathan Walton[2], and Gerald Brosch[1]. [1]Department of Microbiology, Medical School, University of Innsbruck, A-6020 Innsbruck, Austria. [2]Department of Energy-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA.

One basic mechanism of gene regulation in eukaryotic cells is the modification of chromatin structure. Two groups of enzymes, acetyltransferases (HATs) and deacetylases (HDACs), regulate transcription via alternating the acetylation state of histones or promoter-bound transcription factors. Several chemical agents are known to specifically inhibit HDACs. Among them is the cyclic tetrapeptide HC-toxin, which is a secondary metabolite of the maize pathogen fungus *Cochliobolus carbonum*. Since inhibition of HDACs has been shown to cause remarkable hyperacetylation of histones and thereby affects cell cycle progression and differentiation patterns of different sources, we posed the question of how the fungus protects its own chromatin structure against rearrangements caused by hyperacetylated histones. To investigate how the acetylation state of the histones is kept in balance in the presence of HC-toxin, we are analyzing the fungal HDACs. Here we report the cloning of three HDAC genes of

Cochliobolus carbonum, all of which share striking homology to either RPD3 or HDA1 HDACs of *Saccharomyces cerevisiae* . We investigated the influence of inhibitors on partially purified HDAC-activity and identified one inhibitor sensitive and one resistent activity. Western blot analysis with antibodies against the identified enzymes revealed each of them as part of the sensitive activity. Furthermore, we used immunoprecipitation experiments to proof the in vivo HDAC-activity of the identified proteins.

340 **Characterization of PA-specific incompatibility factors in the *het-6* region of *Neurospora crassa*.** Cristina O. Micali and M. L. Smith. Carleton University, Ottawa, ON. Canada

In *N. crassa*, only individuals carrying the same alleles at all *het* loci are able to fuse and grow vegetatively as heterokaryons. The *het-6* region in *N. crassa* is located on the left arm of linkage group II and carries two closely linked *het* genes. *un-24* encodes the large subunit of ribonucleotide reductase, and *het-6*, encodes a ~680 amino acid protein with similarity to several putative genes in *N. crassa*, including *tol*, and to *het-e* from *Podospora anserina*. Two alleles, designated Oakridge (OR) and Panama (PA), occur at *un-24* and *het-6*. Incompatibility activity of the OR forms was previously described. Here, we report the partial characterization of the PA-specific incompatibility activity associated with *un-24* and *het-6*. Unlike the OR counterparts which cause severe incompatibility reactions, transformation of *het-6*^{PA} into OR spheroplasts results in a moderate reduction in the number of transformants, while *un-24*^{PA} causes growth inhibition and a "spidery" colony morphology in transformed OR colonies, similar to the *het-c* and *mat* incompatibility phenotypes. Escape from *un-24*PA/OR self-incompatibility occurs within 2 to 8 days to give near wild-type growing colonies. Preliminary data indicate that while crosses between escapes and *het-6*^{OR} strains are fertile, escape x *het-6*^{PA} crosses are barren. This suggests that OR/PA and PA/OR combinations of *un-24/het-6*, which are not observed in nature, may be selected against during the sexual cycle.

341 **Characterization of a transcription factor that regulates HC-toxin production in *Cochliobolus carbonum*.** Kerry F. Pedley and Jonathan D. Walton. DOE-Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Cochliobolus carbonum is the causal agent of northern corn leaf spot. Strains of the fungus that produce a host-selective toxin, HC-toxin, are highly virulent towards certain genotypes of *Zea mays*. HC-toxin is a non-ribosomally synthesized cyclic tetrapeptide with the structure (D-Pro-L-Ala-D-Ala-L-Aeo), where Aeo stands for 2-amino-9,10-epoxi-8-oxodecanoic acid. Production of HC-toxin is under the control of a single genetic locus, *TOX2*. This locus is composed of at least six linked genes; *HTS1, TOXA, TOXC, TOXE, TOXF, and TOXG*. The products of these genes have demonstrated or probable roles in the biosynthesis of HC-toxin. *TOXE* has been shown to be required for the expression of the known genes within the *TOX2* locus. DNA sequence analysis of *TOXE* shows that its predicted product (TOXEp) contains a basic region similar to those of basic leucine zipper (bZIP) proteins, but lacks a leucine zipper dimerization motif. TOXEp also contains four ankyrin repeats. We have been testing the hypothesis that TOXEp is directly involved in the transcriptional regulation of the HC-toxin biosynthetic genes. Although TOXEp does not contain a well-conserved DNA binding domain, we have demonstrated that TOXEp directly interacts with conserved elements in the promoters of the genes within the *TOX2* locus. Additionally, we have shown that TOXEp is a strong activator of transcription and is capable of acting as a transcription factor in yeast. We believe TOXEp is a unique pathway-specific transcription factor that is responsible for regulating the expression of the *TOX2* locus. Thus, it plays a key role in HC-toxin production and pathogenicity.

342 **How did such a large number of B mating types of *Schizophyllum commune* evolve in nature?** Thomas J. Fowler, Michael F. Mitton, Cynthia L. St. Hilaire, and Carlene A. Raper. Department of Microbiology and Molecular Genetics, University of Vermont, Burlington VT 05405 USA.

The Homobasidiomycetous fungus *Schizophyllum commune*has thousands of mating types defined in part by numerous lipopeptide pheromones and their G protein-linked receptors encoded within different versions of two redundantly functioning B mating-type loci, B-alpha and B-beta. Compatible combinations of pheromones and receptors produced by individuals of different B mating types regulate a pathway of sexual development required for establishment and maintenance of the dikaryon. Sequence comparisons of the B-alpha1 and B-beta1 loci indicate that these two loci may have been derived from a common ancestor by duplication and inversion. Recent molecular genetic analyses of another specificity of the B-beta locus, B-beta2, revealed a single pheromone receptor gene and a surprisingly large number of pheromone genes, four of which are similar in sequence and appear to have been

derived by duplication. Two of these activate the identical set of receptors encoded by other B-beta specificities; the other two may be pseudogenes. This evidence of gene duplication, together with our recent demonstration that small changes in amino acid sequence of both pheromones and receptors can significantly alter specificity of pheromone/ receptor interactions, has provided clues about how so many different versions of the B mating-type loci could have evolved in nature. A multi-step model involving tandem gene duplication and mutational divergence will be discussed.

343 **Transformation of the oomycete, *Phytophthora infestans*, using microprojectile bombardment.** Cristina Cvitanich and Howard S. Judelson. Department of Plant Pathology, University of California, Riverside USA

Optimal transformation procedures for *P. infestans*, the potato late blight pathogen, will assist the analysis of genes relevant to its growth and pathogenesis and thus facilitate developing disease control strategies. A reliable method for transforming *P. infestans* protoplasts was developed in our laboratory and is being widely employed. However, that protocol involves generating protoplasts using Novozyme 234, an enzyme which is no longer produced and was of variable quality. As an alternative to the protoplast protocol, microprojectile bombardment is being optimized using a helium-driven system (PDS- 1000/He, Biorad). For these experiments, the pTH209-35G vector, which expresses the *neomycin phosphotransferase (npt)* and *beta-glucuronidase(gus)* genes, is being used to transform tissue which is then subjected to selection using G418- supplemented media. Transformants are also assayed histochemically for *gus* expression. To optimize transformation, variables tested included helium pressures between 450 and 1800 psi, different particle flight distances, treating mycelia versus germinated asexual spores, different concentrations and ages of the target tissue, and various selection schemes Using the optimal conditions up to 16 G418-resistant colonies were recovered per bombardment event, employing 1 microgram DNA per shot. The majority of transformants appeared three to four days after transformation, and data so far indicates that one to two copies of the plasmid normally integrate into the genome. The levels of *gus* expression varied between independent transformants, probably due to position effects. Overall, microprojectile bombardment is a suitable method for transforming *P. infestans* which avoids protoplasting and shows low experiment-to-experiment variation.

344 **Molecular mechanism responsible for the dominant repression of aflatoxin biosynthesis in *Aspergillus flavus.*** Gyung-Hye Huh, Joe Flaherty, and Charles Woloshuk. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN

Biosynthesis of aflatoxin, a toxic and carcinogenic secondary metabolites, is by the polyketide pathway of secondary mechanism. Expression of genes in the aflatoxin biosynthesis cluster is regulated by *aflR*. *A. flavus* strain 649, mutated at the *afl-1* locus, has a genomic DNA deletion greater than 150 kb covering the aflatoxin biosynthesis cluster. Diploids formed by parasexual cross between 649 and aflatoxigenic strains do not produce aflatoxin indicating the dominant phenotype associated with the *afl-1* locus in strain 649. We have hypothesized that the dominant suppression phenotype arises by one of two possible mechanisms: i) inactivation of alleles due to homologous pairing of chromosomes (transvection), ii) the suppression of aflatoxin biosynthesis by the aberrant expression of a repressor gene in strain 649. Using a unique band obtained by AFLP analysis, we have isolated a cosmid that appears to contain DNA from the break-junction region in strain 649. Characterization of this cosmid will determine if there is an insertion of interfering DNA at the break-junction or if the two ends of the deletion are simply ligated. To address the possibility of a suppressor, a vector having *aflR* and *ver1*::GUS reporter gene was inserted into the 649 genome. We are currently screening for transformants with a single copy insertion. We will present measurements of GUS expression, aflatoxin production, and expression of aflatoxin biosynthesis genes in the transformants and diploids between transformants and an aflatoxigenic strain. Results from this study will provide us with a clearer understanding of the afl-1 locus in strain 649.

345 **Characterization of transposable elements in *Cryptococcus neoformans.*** M. Cristina Cruz[1] and Joseph. Heitman[1- 2], Department of Genetics,Duke University Medical Center[1] and The Howard Hughes Medical Institute[2], Durham NC 27710 USA

Cryptococcus neoformans is a fungal pathogen that causes life-threatening central nervous system infections in immunocompromised patients. Transposons are genetic elements that are distributed throughout the genomes of organisms from bacteria to humans and that are capable of moving within their host. We have identified novel insertion sequences in *C. neoformans*that represent the first transposable elements to be described in this human

pathogen. Of four spontaneous FK506-resistant mutants that were previously isolated, two were found to result from the insertion of novel DNA sequences into the *FRR1*locus encoding the FK506 target protein FKBP12. Isolation and sequence analysis revealed that these two novel elements are flanked by inverted repeats and created either a five-bp or a three-bp target site duplication upon insertion. These features suggest that these insertion sequences represent novel transposable elements. One element is 2749 bp and the second element is 785 bp. Southern blot analysis revealed multiple genomic DNA fragments that hybridize with each element in the two congenic serotype D strains JEC20 and JEC21 as well as in their parental strains. In summary, we have identified two novel tranposable elements that are found in moderate copy number in the genome of C. neoformans serotype D strains and which are infrequent or absent in serotype A, B, or C strains. This discovery marks the first transposable elements to be described in a pathogenic basidiomycete and will allow studies on their possible roles in virulence, evolution, phenotypic switching, and their use in novel approaches to manipulate and mutagenize the genome.

346 **Role of F-box proteins (Fbls) in filamentous fungi.** Janna Beckerman, Carlos Cortes, Cristina Filippi, Jim Sweigard, Barbara Valent and Daniel Ebbole. Plant Pathology & Microbiology, Texas A&M University, College Station, TX.

F-box proteins are components of modular E3 ubiquitin protein ligases called SCF's, which direct protein ubiquitination. The SCF system controls a large number of regulatory pathways in vertebrates. In *Saccharomyces cerevisiae*, the F-box protein Grr1 appears to play a key role in cell cycle progression and response to glucose availability. The expression of yeast glucose transporters is under control of this F-box protein. To further understand the role of SCF systems in eukaryotic cells, we are characterizing two genes encoding F-box proteins from *Magnaporthe grisea*(*pth1*) and *Neurospora crassa* (*nfb1*). The deduced sequence from these proteins display extensive internal homology within the leucine-rich repeat and are most closely related to Grr1p. *M. grisea*, a grass pathogen, undergoes appressorium development to establish a successful host-parasite interaction. Mutants of *pth1* produce defective appressoria that are sensitive to hyperosmotic stress. Thus, *pth1* mutants are defective in the penetration process. Typical lesions were only observed after wound inoculations of *pth1* mutants on rice and barley plants, suggesting that disruption of this gene does not affect pathogenicity but does interfere with the penetration of plant tissue. No other difference has been detected among wild type and *pth1* mutants. Phenotypic analysis of *nfb1* in *N. crassa* will provide additional clues about the role of F-box proteins in filamentous fungi.

347 **Mitochondrial nuclear communication in *Neurospora crassa.*** Andrea T. Descheneau, Ian A. Cleary, Frank E. Nargang. University of Alberta, Biological Sciences, Edmonton, Alberta, Canada

Mitochondria are the site of oxidative phosphorylation, a key energy generating process in most eukaryotic cells. Although some mitochondrial proteins are encoded by the mitochondrial genome, the majority are encoded in the nucleus. Some form of communication is believed to exist between these two organelles to coordinate their gene expression, thereby insuring proper mitochondrial biogenesis and function. However, the nature of this communication remains unknown. In *Neurospora crassa*, deficiencies in oxidative phosphorylation result in expression of several nuclear genes. One of these genes, *aod- 1*, is only expressed during times of respiratory stress and encodes an alternative oxidase that is able to pass electrons directly from ubiquinone to oxygen. We are interested in understanding how mitochondria signal the nucleus to induce alternative oxidase expression. A reporter has been constructed using the alternative oxidase promoter sequence and the structural gene for the melanogenesis enzyme tyrosinase. Mutagenesis of a reporter strain has allowed the isolation of mutant strains that are altered in their ability to regulate both reporter and alternative oxidase gene expression. Further characterization of these mutants may be useful in determining the pathway responsible for alternative oxidase regulation.
Acknowledgements: This work was supported by studentships from the Alberta Heritage Foundation for Medical Research and the Natural Sciences and Engineering Research Council of Canada to ATD, and a grant from the Natural Sciences and Engineering Research Council of Canada to FEN.

348 **Understanding genetic mechanisms involved in fumonisin biosynthesis.** J.E. Flaherty, W-B Shim and C.P. Woloshuk. Purdue University, West Lafayette, IN

Fumonisins are a group of naturally occurring metabolites produced by *Gibberella fujikoroi* (anamorph *Fusarium verticillioides*), which frequently contaminate corn-based foodstuffs and animal feed. The B series of fumonisins (FB1) is the most prevalent and has been implicated as the cause of several animal toxicoses as well as cancer

promotion in rats and humans. The objective of our research is to clone and characterize genes involved in fumonisin gene regulation and to set the context in which regulation of the fumonisin biosynthetic pathway exists in relation to other developmental and/or physiological cascades. By the random enzyme-mediated integration (REMI) technique, we isolated *fic1* from *G. fujikuroi*, a type-C cyclin similar to UME3 in *Saccharomyces cerevisea*. Disruption mutants of *fic1* fail to produce fumonisin and exhibit reduced conidiation when grown on corn. We have isolated ESTs (>1500) differentially transcribed in a wild-type fumonisin producing strain compared to a *fic1*disruptant cultured on corn. Sequence analysis of 800 ESTs revealed transcripts with high sequence homology (> 1E-5) to known regulatory genes, including transcription factors, kinases and cyclin/CDKs. Toward understanding the functional role of these genes, we are constructing knockout vectors for specific disruption via homologous recombination. We will describe the phenotypes associated with the disruption events in *G. fujikuroi* and determine their effects on fumonisin biosynthesis and fungal development. We will also present analysis of new REMI transformants generated with a reporter construct containing the promoter region of *fum5*, a known fumonisin pathway gene, fused to *uidA* (GUS). Appearance of GUS activity parallels that of fumonisin accumulation in culture; therefore, mutations affecting *fum5* transcription should also alter fumonisin biosynthesis.

349 **Expression analysis of *Phanerochaete chrysosporium* cellulase and lignin peroxidase genes using RT-PCR and DNA microarrays techniques.** Debbie Yaver[1], Daniel Cullen [2, 3], Jennifer Bassett[3], Amber Vanden Wymelenberg[3], Xiaochun Yu [4], Rajai Atalla [2,4]. [1] Novozymes Biotech, Davis, CA, [2] USDA Forest Products Lab, Madison, WI, [3] Dept. of Bacteriology, University of Wisconsin-Madison, Madison, WI, [4] Dept. of Chemical Engineering, University of Wisconisin-Madison, Madison, WI.

Transcript levels of cellobiohydrolase, cellobiose dehydrogenase, and beta-glucosidase genes were quantified for *Phanerochaete chrysosporium* grown on basal salts containing either Avicel or CF-1 cellulose as the sole carbon source. Differential regulation was observed in response to secondary and/or tertiary structures of cellulose substrates and over time. Competitive RT-PCR techniques and DNA microarrays were in general agreement regarding the relative expression of six structurally related cbh1 genes. In addition, expression analysis of the lignin peroxidase genes in cultures grown in complete, carbon limited and nitrogen limited medium was performed using DNA microarrays. The results with DNA microarrays and prior studies with competitive RT-PCR were comparable. Microarray approaches hold considerable promise for rapid and convenient transcript profiling, especially within families of closely related genes.

350 **Isolation and characterisation of two ammonium permeases in *Aspergillus nidulans.*** Brendon J. Monahan, Michael J. Hynes and Meryl A. Davis. Department of Genetics, University of Melbourne, Victoria 3010, Australia.

In filamentous fungi and yeast the preferential utilisation of favoured nitrogen sources such as ammonium and glutamine, termed nitrogen metabolite repression (NMR), is a dynamic system capable of responding to changes in the nitrogen status of the cell. NMR in *A. nidulans* is mediated by *areA*, a positively acting gene whose product, the GATAûûtranscription factor AreA, is required to alleviate NMR with *areA* loss of function mutants characterised by their inability to grow on non-preferred nitrogen sources. Because of the central role ammonium plays in the control of NMR, it is important to gain an understanding of the genes and proteins involved in ammonium uptake and their regulation. The presence of at least two specific ammonium transporters has been indicated previously for *A. nidulans*, including the isolation of methylammonium resistant *meaA* mutants which are defective in ammonium transport. Here we present the molecular, kinetic and physiological characterisation of two ammonium permeases, MeaA and MepA from *A. nidulans. meaA* was isolated by complementation of the *meaA8* mutant and *mepA* was isolated by degenerate PCR. Both genes belong to the well characterised MEP/AMT family of ammonium transporters with *meaA* being most similar both in sequence and function to *Mep1* from yeast, and *mepA* most similar to *Mep2.* Deletion strains for both genes have been generated. The double deletion strain displays reduced growth on low ammonium concentrations compared to wild-type. Expression analysis indicates that *mepA* and *meaA* have differing expression patterns but both are regulated by *areA* and to a lesser extent *tamA.*

351 **A novel *Aspergillus nidulans* serine/threonine protein kinase gene that is transcriptionally regulated by camptothecin.** Marcelo A. Vallim; Camile P. Semighini; Renata C. Pascon; Maria Helena S. Goldman & Gustavo H. Goldman. Universidade de São Paulo, Faculdade de Ciências Farmacêuticas de Ribeirão Preto. Av. do Café, S/N. 14040-903 - Ribeirão Preto-SP Brazil

The DNA damage response is a protective mechanism that ensures the maintenance of genomic integrity during cellular reproduction. It consists of extensive repair systems that deal directly with DNA damage, as well as surveillance systems, known as checkpoints, which arrest the cell cycle and provide time for DNA repair before the critical events of replication and segregation occur. Camptothecin (CPT) is an anti-cancer drug that targets eukaryotic DNA topoisomerase I by trapping the covalent complex formed by this enzyme and DNA. Damage to DNA occurs when the replication fork encounters the ternary complex (DNA-topoisomerase I-camptothecin) causing double-strand break and in higher eukaryotes, apoptosis. The filamentous fungus *Aspergillus nidulans* is a biological model to study cell cycle control and DNA damage response, however the mechanism how it responds to double-strand breaks caused by CPT is unknown. In order to uncover genes that might be involved in providing the response to damage to DNA in this fungus, Differential Display Reverse Transcription PCR technique (DD-RTPCR) was employed. Using this method we describe the isolation and characterization of a gene that encodes a novel putative serine/threonine kinase (anpk). Quantitative real-time RT-PCR showed that the transcription of anpk fluctuates during an 8 hours period. However, when camptothecin is added to the medium the anpk transcription is repressed showing its lowest accumulation at 1 hour after CPT induction whereas at 8 hours the drug induced anpk transcription. These results suggest that this kinase might be involved in the mechanism by which A. nidulans responds to DNA damage. This project is sponsored by Fundação de Amparo a Pesquisa do Estado de São to M.A. Vallim (#00/01088-9)

352 **Isolation and characterization of a factor that modulates the gene expression in Aspergillus nidulans.** Balan, A., and Zucchi, T.M.D.A. Department of Parasitology - Instituto de Ciencias Biomedicas II - Universidade de Sao Paulo - Brasil.

The su30 strain is a mutant of *A. nidulans* that modulates the gene expression of several genes. This strain when in contact with the UT196, that is deficient to methionine synthesis (met) and pyridoxin (pyro), is able to revert this phenotypes for met+ and piro+. Crosses and heterocaria analysis showed the presence of the factor (Fsu30) difusible onto citoplasm with activity in the mitosis and meiosis. Strains submitted to the treatment with the supernatant of Su30 presented different phenotypes and protein profile. The modified characters had shown high stability. Differences between the strains UT448 and su30 had been evidenced through SDS-PAGE. The supernatant of su30 also promoted alterations in the profile of restriction of plasmid DNA when submitted to the restriction enzymes. Hibridization analysis have showed that the rDNA fragment is methylated in *Aspergillus nidulans*, but the Fsu30 doesn't promoted any alteration in this sequence.

Financial Support: FAPESP

353 **Photoactivation of gene expression in Phycomyces and Neurospora.** Luis M. Corrochano, Lucia Bautista, Manuel Castellano-Munoz, Rocio P. Cotarelo, Maria Perez-Caro and Charles Yanofsky[1]. Departamento de Genetica, Universidad de Sevilla, Sevilla, Spain. [1]Department of Biological Sciences, Stanford University

The Zygomycete *Phycomyces blakesleeanus* develops two types of sporangiophores of very different size: macrophores and microphores. Blue light inhibits microphorogenesis and stimulates macrophorogenesis. We have isolated a segment of the gene encoding the heat-shock protein HSP100 which is induced at the onset of sporulation. The effect of mutations on the expression of this gene and the isolation of its regulatory sequences will aid to establish the molecular details of photomorphogenesis in Phycomyces. The gene con-10 of Neurospora is expressed during conidiation and following illumination of mycelia with light. The photoactivation of con-10 disappears after two hours of illumination (light adaptation). We have designed a protocol to isolate mutants altered in the adaptation of con-10 photoactivation. We are using a strain of Neurospora with a fusion of the con-10 promoter to the gene conferring resistance to hygromycin. This strain is sensitive to the drug when the promoter is inactive, i.e. during vegetative growth either in the dark or under continuous light. We have isolated three mutants that grow in the presence of hygromycin under continuous light but not in the dark. Presumably this is due to a defect in the mechanism controlling light adaptation.

354 **The NIT2 and NIT4 regulatory proteins must cooperate to activate gene expression in *Neurospora crassa*.** Xiao-kui Mo and George A. Marzluf. Ohio State University, Biochemistry, Columbus, Ohio, USA

In *Neurospora crassa*, the nit-3 gene, which encodes nitrate reductase, an enzyme required for the utilization of inorganic nitrate, is subject to a high degree of genetic and metabolic regulation. The nit-3 gene promoter contains binding sites for the globally-acting NIT2 protein and for NIT4, a pathway-specific transcription factor. Expression of the nit-3 gene absolutely requires both the NIT2 and NIT4 proteins and only occurs under conditions of nitrogen source derepression and nitrate induction. The cys-14 gene encodes sulfate permease II, which facilitates the assimilation of sulfate. Expression of cys-14 is strongly regulated and its activation requires only a single positive-acting factor, CYS3, a bZip protein. It was of interest to determine whether NIT2 or NIT4 alone would be capable of turning on expression of cys-14 since this structural gene is normally controlled by only one regulatory protein. NIT2 and/or NIT4 binding elements were introduced into the cys-14 promoter and these constructs were transformed into a cys-13, cys-14 strain and also into nit-2 and nit-4 mutant hosts. The cys-14 gene in these transformants could now be controlled as a nitrogen-regulated gene. Sulfate permease assays revealed that both NIT2 and NIT4 were required for cys-14 expression during nitrogen induction conditions. Neither alone was able to activate any detectable cys-14 expression. Thus, we conclude that NIT2 and NIT4 are neither capable alone of activating gene expression in this context, but together can cooperate to elicit strong activation. The strain containing the cys-14 gene armed with both NIT2 and NIT4 binding elements was still able to respond to sulfur derepression. Thus this redesigned cys-14 gene is regulated by both nitrogen and sulfur control systems. When derepressed for both nitrogen and sulfur, a synergy was observed and cys-14 was expressed at a much greater level than expected by a simple additive effect.

355 ***Fopta1*, a trascriptional activator specific to highly virulent strains of *Fusarium oxysporum* f.sp. *phaseoli.*** B. Ramos[1], F.M. Alves-Santos[2], M. Perlin[1], E.A. Iturriaga[1], R. Martín-Domínguez[1], M.A. García-Sánchez[1], A.P. Eslava[1] and J.M. Diaz-Minguez[1]. [1]Area de Genitica, Centro Hispano-Luso de Investigaciones Agrarias, Universidad de Salamanca, 37900 Salamanca, Spain. [2]Plant Breeding Department, MBG-CSIC, 36080

Pontevedra, Spain.

The plant pathogen *Fusarium oxysporum* is a common soilborne fungus with a worldwide distribution. Within the species there is a high level of host specificity with over 120 described formae speciales and races capable of causing vascular wilt diseases of many agricultural crops. This combination of wide range of infection as species and host specificity as formae speciales makes *F. oxysporum* an attractive model for the study of the molecular interactions involved in pathogenicity and/or virulence. Fusarium wilt, caused by *F. oxyspourm* f. sp. *phaseoli* J.B. Kendrick & W.C. Snyder, is a serious disease of common bean (*Phaseolus vulgaris* L.). In the course of the genetic characterization by RAPD analysis of *F. oxysporum* f.sp. *phaseoli*, we have isolated a gene, *Fopta1*, specific to the highly virulent strains of all known races of the pathogen. Molecular analysis allowed the identification of *Fopta1* as a transcriptional activator belonging to the Gal4 family, which is characterized by the presence of the C_6 Zinc finger motif. Expression of *Fopta1* has been detected by RT-PCR both in mycelium of highly virulent strains grown in vitro and in bean plants artificially inoculated with these strains, but its expression was not found in mycelia of nonpathogenic strains. Gene inactivation experiments and a fine analysis of in vivo expression are underway in order to elucidate whether *Fopta1* is a virulence or a pathogenicity factor, and in which mechanisms this gene is involved.

356 **Sugar sensing and regulation of conidiation in *Neurospora***. Xin Xie and Daniel J. Ebbole. Texas A&M University, College Station, TX.

Sensing the presence of a preferred carbon source is a fundamentally important part of the cellular response to the environment. Unfortunately, the pathways for signaling carbon availability in filamentous fungi are poorly understood. In yeast, several mechanisms are involved in glucose sensing and controlling gene expression. These mechanisms include the use of special glucose transporter homologs that have evolved as glucose receptor/sensors but are not themselves functional glucose transporters. The *N. crassa rco-3* mutant conidiates in liquid culture in the presence of carbon sources that repress conidiation in the wild type. The mutant is altered in the activities of multiple sugar uptake systems, is defective in carbon catabolite repression, and is resistant to 2- deoxyglucose and sorbose. The *rco-3* gene is a member of the sugar transporter superfamily with greatest similarity to fungal glucose transporters. Our characterization of *rco-3* suggests it functions as a sugar sensor rather than a sugar transporter. To gain further evidence for the role of *rco-3* as a regulator we have isolates several suppressors of *rco-3* and are examining the effect of the *rco-3* mutant on gene expression. The characterization of the suppressor mutants and

transcriptional profiling of the *rco-3* mutant strain should help us define the genetic pathway involving *rco-3* and the role of *rco-3* in sugar sensing.

357 **Coiled coil domain mediated FRQ-FRQ interaction is essential for its circadian clock function in Neurospora.** Ping Cheng[1], Yuhong Yang[1], Christian Heintzen [2], and Yi Liu [1 1]. Department of Physiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX. 75390-9040 USA [2]. Department of Genetics, Dartmouth Medical School, Hanover, NH. 03755 USA

The frequency (frq) gene, the central component of the frq based circadian negative feedback loop, regulates various aspects of the circadian clock in Neurospora. However, the biochemical function of its protein products, FRQ, is poorly understood. In this study, we demonstrated that the most conserved region of FRQ forms a coiled-coil domain. FRQ interacts with itself in vivo, and the deletion of the coiled-coil region results in the loss of the interaction. Point mutations, which are designed to disrupt the coiled-coil structure, weaken or completely abolish the FRQ self-association and lead to the arrhythmicity of the overt rhythm. Mutations of the FRQ coiled-coil which inhibit self-association, also prevent its interaction with two other key components of the Neurospora circadian clock, namely WC-1 and WC-2, the two PAS domain containing transcription factors. Taken together, these data strongly suggest that the formation of the FRQ-FRQ and FRQ-WC complexes are essential for the function of the Neurospora clock.

358 **Induction of alternative oxidase gene expression by nitric oxide in *Histoplasma capsulatum*.** Joan E. McEwen and Clayton H. Johnson. Central Arkansas VA and Univ. of AR Med. Sci., GRECC and Geriatrics, Little Rock, AR, USA.

Most fungi possess a branched mitochondrial respiratory pathway. Cytochrome c oxidase and alternative oxidase terminate the two branches of the pathway. Both enzymes transfer electrons from an electron donor to dioxygen, with production of harmless water as the product. Because alternative oxidase is absent in mammals, it will be an attractive target for development of antifungal drugs if it is demonstrated to be important for virulence of pathogenic fungi. We hypothesize that alternative oxidase is especially important during pathogenesis, when host antifungal efforts involving nitric oxide or other environmental stresses may inhibit the cytochrome pathway. In support of this hypothesis, experiments on the effect of nitric oxide on *H. capsulatum* mitochondrial function showed rapid inhibition of cytochrome oxidase, but little inhibition of alternative oxidase, by physiological levels (10 to 50 micromolar) of nitric oxide donor. Also, within an hour after nitric oxide exposure, alternative oxidase enzymatic activity and mRNA levels were significantly increased relative to the control, indicating that nitric oxide induces alternative oxidase gene expression. To examine the role of transcriptional induction in this process, a 14 kb genomic fragment containing the *H. capsulatum AOX1* gene for alternative oxidase has been isolated and will be used to dissect the 5' flanking region of the gene for potential transcriptional control elements. Also, a polyclonal antiserum against *H. capsulatum* alternative oxidase has been raised and will aid us in studies of alternative oxidase function and regulation in this fungal pathogen.

359 **Differential display and analysis of sterol induced changes in Phytophthora gene expression.** David W. Dotson, Kevin V. Shianna, Shirley Tove, Leo W. Parks. North Carolina State Unviersity, Microbiology, Raleigh, NC

Although sterols are universally observed as critical membrane components among the eukaryotes, sterol auxotrophy is by no means uncommon. Nevertheless in almost all instances these auxotrophs require an exogenous supply of sterol for viability. Species of *Phytophthora* are exceptional in that their inability to synthesize sterols is combined with a rare ability to grow even in the complete absence of sterols. While these features have proven enticing towards research in sterol metabolism, the genetics of *Phytophthora* remain largely unresolved and experimentally intractable. In spite of this we have demonstrated the capacity of *Phytophthora* to differentially regulate gene expression in response to sterols, and enzymatically modify particular features of sterol nuclei. In this study we have used a differential display (DDRT-PCR) method to isolate cDNA fragments corresponding to genes of *P. cactorum* that are regulated in response to sterol feeding. Sequence analysis has allowed us to identify similar open reading frames in the model eukaryote *Saccharomyces cerevisiae*. In contrast to *Phytophthora*, genetics and biochemistry of *Saccharomyces* are more facile, allowing our pursuit towards a functional characterization for the proteins encoded by the isolated cDNAs. These data should provide insight and new avenues for research into the

functional sterol metabolism of *Saccharomyces* and *Phytophthora*, along with other taxa both fungal and non-fungal alike.

360 **Regulation of fatty acid desaturase gene expression in the oleaginous fungus *Mortierella alpina.*** A.T. Carter , D.A. MacKenzie , P. Wongwathanarat and D.B. Archer. Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK, Dept. of Biotechnology, Thammasat University, Patumthanee12121, Thailand, School of Life and Environmental Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK

M. alpina produces up to 50% of its cell dry weight as triacylglycerol rich in long-chain polyunsaturated fatty acids which are of nutritional and pharmacological importance. Several genes encoding fatty acid desaturases have been isolated from this fungus and we have examined their differential expression during growth in media with high and low C:N ratios. Transcripts from the delta9-fatty acid desaturase gene (*ole1*), the delta6-fatty acid desaturase gene and the acetyl-CoA carboxylase gene were detected in mycelium grown in rich, low C:N medium and showed increased levels during growth in high C:N medium in which oil accumulation is stimulated. Transcription of a second delta9-fatty acid desaturase gene (*ole2*) and of a third, putative fatty acid desaturase gene (delta9-3) was negligible when the fungus was grown in low C:N medium but was induced significantly in high C:N medium. Reduction of growth temperature from 25 C to 12 C also caused a significant increase in mRNA levels for all three delta9-like genes. The delta9-3 gene failed to complement the yeast *ole1* mutation unlike the *M. alpina ole1* and *ole2* genes. Fatty acid analysis of yeast transformants indicated that the delta9-3 gene product desaturated hexacosanoic acid (26:0) to hexacosenoic acid (26:1 n-9), a reaction which the *ole1* and *ole2* enzymes also carried out. Hexacosanoic acid is not found in *M. alpina* and the true substrate for delta9-3 remains to be elucidated.

361 **The catalase isozymes and genes of *Histoplasma capsulatum.*** Clayton Johnson, Joan McEwen, Lyndal York. Univ. of AR Med. Sci. and Central Arkansas VA, Geriatrics and GRECC Little Rock, AR

The dimorphic fungus, *H. capsulatum*, encounters significant environmental stress during its life cycles. To uncover its oxidative stress response pathways, we isolated three catalase cDNAs (*CATA*, *CATB*, and *CATP*) and a *CATA* genomic fragment. We purified a *de novo* or recombinant forms of 2 of these enzymes and used them to raise antisera. We are using these tools to characterize the expression levels and cellular locations of the products of these genes. Finally, we are using the catalase clones in attempts to generate knockout mutants.

Our immunoblots confirm that catalase P is a soluble enzyme that is likely to be peroxisomal. Our preliminary catalase A immunoblots indicate this enzyme is extremely insoluble and localized at the cellular surface. Northern blot results show different patterns of expression of the 3 catalase genes. Abundant transcripts for both *CATB* and *CATP* were detected under all growth conditions examined, but a modest decrease in RNA abundance was observed for each gene after growth of either yeast or mycelial cells in medium containing glucose rather than glycerol as carbon source. In contrast, *CATA* RNA levels were nearly undetectable under most conditions of growth, but showed marked elevation after oxidative stress.

Our work confirms a significant redundancy in cellular catalase function and is interesting in light of the role of *H. capsulatum* as an intracellular pathogen that may be exposed to high levels of oxidative stress during infection of a mammalian host. Gene knockouts, when available, will elucidate the different roles of each catalase isozyme.

362 **Aspects of gene regulation by ambient pH in *Aspergillus nidulans.*** Joan Tilburn. Imperial College School of Medicine, Infectious Diseases, London, UK

Response to ambient pH is mediated by the PacC transcription factor. Under conditions of alkaline ambient pH, PacC is proteolysed to its active form in response to a signal mediated by the *pal* gene pathway. This carboxy-terminally truncated PacC form is an activator of alkaline expressed genes and, in conjunction with the *pacM* gene product, a repressor of acid expressed genes. pH regulation in *A. nidulans* affects levels of gene products of commercial and/or health importance and studies here underpin work in other organisms where homologues of components of the system are involved in virulence determination and/or sex. Aspects of our current understanding of pH regulation will be presented.

363 **Heterologous expression of human insulin by *Aspergillus nidulans.*** Jesus, K.R.E; Meilus, M.* & Zucchi, T.M.D.A.*. Universidade de Sao Paulo - PostGraduate Program of Biotechnology. *Universidade de Sao Paulo - Department of Parasitology

Filamentous fungi of genus Aspergillus have been intensively used as receptor strain to express heterologous protein mainly due to their ability of secreting high amounts of extra cell proteins in a very inexpensive way. In the present case, A. nidulans was chosen on account of its well known genetic background, allied to good possibilities of producing transformants from protoplasts. Besides, cultures are of easy preparation, and even overnight experiments can yield large amounts of secreted proteins. After RNA treatment (Zucchi, 1996), several human insulin expressing Aspergillus strains were obtained. Despite high meiotic instability, meiotic and mitotic crosses produced several stable strains which were selected after their ability of expressing high insulin amounts. High instability presented by the RNA treated strains, allied to changes in the expression of resident genes evidence insertions, what may indicate the triggering of genomic defense against exogenous insertions. The control over instabilities promoted by genomic defense against DNA invaders, constitutes the main focus of this research.

364 **Photoregulation of carotenoid biosynthesis in *Mucor.*** Enrique A. Iturriaga, Antonio Velayos, and Arturo P. Eslava. Area de Gen tica, Departamento de Microbiologia y Genetica, Universidad de Salamanca, Salamanca, Spain

The biosynthesis of carotenoids in the zygomycete *Mucor circinelloides* is a process positively regulated by light. Beta-carotene, the main product of the carotenogenesis pathway, is synthesized from geranylgeranyl pyrophosphate after three enzymatic steps: phytoene synthesis, phytoene dehydrogenation and lycopene cyclization. Only two structural genes are involved in this conversion. The *carB* gene is responsible for the phytoene dehydrogenation and the *carRP* gene performs the first and last steps of the pathway. Both genes are arranged in opposite directions, with an intergenic region of 446 bp. Northern analyses from mycelia irradiated with short pulses of blue light showed a fast increase in messenger accumulation. The kinetics of such accumulation was very similar for both genes, showing a typical biphasic response with two maxima in the first twenty minutes after irradiation. The ratio between the maximum level of accumulation and the levels in the darkness control was fluence-dependent, showing a ten-fold increase with 1 Jm^{-2} and more than two hundred-fold with 960 Jm^{-2}. A high rate of mRNA degradation was also detected, what suggests a fast turnover of both messengers, and possibly a mechanism of adaptation. Sequence analysis of the intergenic region showed the presence of several motifs similar to the APE sequences described in the promoters of blue light-regulated genes of *Neurospora crassa*. The fact that the mRNA accumulation levels for both genes in response to light are very similar may reflect a bi-directional mode of action of these APE-like elements.

365 **Interconnected feedback loops in the neurospora circadian system: interactions between wc-1 and frq**. Kwangwon Lee, Jennifer J. Loros, and Jay C. Dunlap. Department of Biochemistry and Department of Genetics, Dartmouth Medical School. Hanover NH. 03755.

Neurospora crassa serves as a excellent model system to identify and characterize the molecular components of the cellular circadian oscillator. Previous work has demonstrated that FRQ and WC-1 are key components of a functional cellular oscillator. The current molecular paradigm for the cellular circadian oscillator includes a transcription/translation based feedback loop composed of negative elements (FRQ in *Neurospora*, PER and TIM in *Drosophila*, and mPER and mCRY families in mammals) and PAS domain containing positive elements (WC-1 and WC-2 in *Neurospora*, dCLK and CYC in *Drosophila*, and CLOCK and BMAL1 in mammals). WC-1 forms heterodimers with its partner WC-2 and transduces the light signal to *frq*. Analyses of a knock-out strain and different mutant alleles of *wc-1* confirmed that WC-1 is necessary for light induction for *frq* and for the robust oscillation of FRQ in constant darkness. WC-1 protein is rhythmic in constant darkness, whereas the steady state level of transcripts are constant. Our data suggest that FRQ is not only a negative regulator of its expression but also a positive regulator for WC-1 through post-transcriptional regulation. We propose that these interdependent regulations of the two key players FRQ and WC-1 in the Neurospora circadian oscillator promotes robustness of the oscillation. Similar interlocked feedback loop structures have been reported in *Drosophila* (Glossop et al. 1999, Science 286:766-768) and the mouse (Shearman et al. 2000, Science 288:1013-1019).

366 **Transcriptional regulation of mating in *Candida albicans* by genes at the mating-type-like locus.** Ryan M. Raisner. UC. San Francisco, Microbiology and Immunol. San Francisco, CA, USA

We have recently discovered a cryptic sexual cycle in *Candida albicans*, a pathogenic yeast previously classified as asexual. *C. albicans* has a mating type like locus encoding nine genes, some of which control mating. We have preliminary evidence that some of these genes are able to control mating through transcriptional regulation.

367 **Genetic and physical analysis of a BAC contig containing the mating type locus of *Phytophthora infestans*.** Audrey M.V. Ah Fong, Thomas A. Randall, and Howard S. Judelson. Department of Plant Pathology, University of California Riverside, California, USA 92521

A map-based cloning scheme is being used to isolate the mating type locus of *P. infestans*, the oomyceteous species that causes the late blight diseases of potato and tomato. *P. infestans* is heterothallic, and exhibits two mating types, A1 and A2. The current model for mating type suggests that the A1 mating type is heterozygous at the mating type locus (*A*/*a*) and the A2 is homozygous (*a*/*a*). Molecular markers B1 (very tightly linked to mating type), AP1 and X1 (which flank B1 at distances of 15 and 13 cM, respectively) were used to isolate clones from a BAC library. These were assembled into contigs representing the *A* and *a*-containing chromosome homologues. Physical mapping of the B1-hybridizing BACs revealed heteromorphic regions between the two contigs. Genetic mapping using cleaved amplified polymorphisms (CAPS) and single strand conformational polymorphisms (SSCP) narrowed the region that contained the mating locus to within 135 kb of DNA between the loci AP1 and X1. cDNAs hybridizing within the contigs were isolated to identify candidate genes and additional markers for mapping. A *Sal*I polymorphism detected by cDNA 637 further delimited the mating type locus to a 60-70 kb region. Additional markers are being identified for further mapping studies and the search for more recombinants in the region will help define the boundaries of the mating type locus.

368 **Functional analysis of FL, a binuclear zinc cluster protein regulating conidiation in *Neurospora*.** Panan Rerngsamran, Xin Xie and Daniel J. Ebbole. Texas A&M University, College Station, TX.

The *fl* gene is necessary for conidiation and, when expressed from a heterologous promoter, *fl* is sufficient to induce conidial morphogenesis. To examine the role of fl in regulating conidiation we have begun a functional analysis of the FL protein. Expression profiling of *N. crassa* genes that depend on *fl* for their expression is being used to identify genes whose promoters may contain binding sites for FL protein. We plan to use affinity purified of 6xHis tagged versions of FL to identify the DNA binding site for FL in these promoters.

369 **Analysis of *cyt*bi3 mitochondrial group I intron ORFs from *Podospora* and *Neurospora* for homing endonuclease activity**. Jill Salvo, Union College, Department of Biology, Schenectady, NY 12308

A number of unusual protein-coding regions exist within the introns of the mitochondrial genes of *Podospora anserina*. Many of these show similarities to known mitochondrial maturases and homing endonucleases (Cummings et al., 1990). The *cyt*bi3 intron ORF shows a 25% identity with the homing endonuclease I Sce I overall, and a 62% identity in the two dodecapeptide repeats, the LAGLI-DADG motif, associated with endonuclease/maturase function. The optional *cyt*bi3 intron of *Podospora* is also optional in *Neurospora* at the same location, and the intron encoded ORFs share extensive similarities (199/229 identical amino acid residues; R. Collins, personal communication). A comparative analysis of these two potential homing endonucleases has been undertaken to establish the extent of similarity with regard to their endonucleolytic activity and substrate specificity. We have used PCR technology to engineer and clone several the *cyt*bi3 intron ORFs into a highly regulated *E. coli* expression vector, which makes use of an intein to provide the final protein product. It was also necessary to alter mitochondrial tryptophan codons (UGA) to the universal tryptophan codon, UGG. Concurrently with the cloning of the ORF, a potential target for the endonucleases was constructed. In general, these sites span from 20 to 60 base pairs around the cleavage site, thus we used an intron-minus allele to obtain as a source for the potential target site. There are a number of interesting evolutionary considerations in regard to the origins of these introns and their ORFs in these organisms, and the possibility of horizontal (between species) gene transfer seems to be plausible, although not yet demonstrable.

370 **A novel bZIP transcription factor in *Neurospora crassa*.** Harriett Bowannie Platero and Mary Anne Nelson. Univ of New Mexico Biology, Albuquerque, NM

A novel gene, tentatively named *zip-1*, has been identified by the *Neurospora* Genome Project. The gene encodes a protein (219 amino acids) that is preferentially expressed during the sexual stage of development of *Neurospora crassa*. The protein contains a DNA-binding domain and an adjacent leucine zipper. These two regions are characteristic of the bZIP family of transcription factors, which includes the mammalian transcription factors *c-jun* and *c-fos*, as well as *GCN4*of *Saccharomyces cerevisiae*. Mutation of the *zip-1* gene results in delayed sexual development. Currently, microarray analysis is being utilized to identify genes directly or indirectly regulated by this novel transcription factor.

371 **Translational suppression of UAG amber nonsense codons in cell-free extracts derived from *Neurospora crassa* supersuppressor mutants**. Peng Fang, Cheng Wu, and Matthew S. Sachs. Oregon Graduate Institute of Science and Technology, 20000 NW Walker Road, Beaverton, OR 97006-8921

Neurospora crassa has ten mapped supersuppressor (*ssu*) genes that suppress the effects of premature termination mutations. *In vivo* studies indicate that they can suppress amber (UAG) codons but the spectrum of their functions remains to be elucidated. We examined suppressor activity using cell-free translation extracts from wild-type *N. crassa* and seven distinct *ssu* strains, *ssu-1*, *-2*, *-3*, *-4*, *- 5*, *-9*, and *-10*. We tested suppression of three kinds of nonsense codons, UAA, UGA, and UAG *in vitro* by requiring suppression to produce functional full-length firefly luciferase enzyme. Luciferase assays showed that all of the *ssu* strains with the exception of *ssu-3* specifically suppressed the amber codon but not the other nonsense codons. Relative to constructs containing sense codons at the corresponding position, enzyme activities produced with UAG varied from 15% to 30%, while activities produced with UAA (ochre) or UGA (opal) at the corresponding position were about 1% to 2%, similar to levels observed with any premature termination codon using wild-type or *ssu-3* extracts. Corresponding results were observed using 35S-methionine labeling to visualize the luciferase polypeptide directly following gel electrophoresis. Finally, using a primer extension-inhibition assay to map ribosomes on the RNA, only when there was suppression were ribosomes observed to continue translation of the reading frame downstream of the nonsense codon.

372 **Cloning of the SIP3 homologue in *Aspergillus nidulans***. Shobana Krishnan, Rosanna Penna-Muralla and Rolf Prade. Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater OK 74078 USA

In *Saccharomyces cerevisiae*, the Snf1 (sucrose non-fermenting) protein kinase is required for transcription of glucose-repressed genes when glucose is limiting. The Sip3 protein belongs to the Snf1 kinase family and was found to interact *"in vitro"* with Snf1. The aim of this research is to isolate the *sip3* homologue in *A. nidulans* and to determine whether *sip3* is involved in its carbon catabolite repression. We have isolated cosmids that hybridize to a *sip3* probe, and the entire genomic region has been sub- cloned and sequenced. The *sip3* probe used is a clone that contains a fragment of the *sip3* homologue in *A. nidulans* rescued in a different study. A *sip3* deletion mutant will be created by transformation-mediated gene replacement and the growth in various carbon-sources like glucose, sucrose and pectin studied and compared to wild type.

373 **PkaA regulates AflR activity by phosphorylation in *Aspergillus nidulans*.** Julie K. Hicks[1], Kiminori Shimizu[2] and Nancy P. Keller[2]. Texas A&M University, Dept. of Plant Pathology & Microbiology, College Station, TX, USA. [1]Present address: Dept. of Biology, Texas A&M University, College Station, TX, USA. [2]Present address: Dept. of Plant Pathology, University of Wisconsin, Madison, WI, USA.

Aspergillus nidulans is a known producer of the carcinogenic mycotoxin, sterigmatocystin (ST). It has been shown that ST biosynthesis is controlled by a G protein mediated signaling pathway. One identified component of this pathway is PkaA, a catalytic subunit of a cAMP- dependent protein kinase (PKA). Previous work indicates that PkaA negatively regulates AflR, a transcription factor that positively regulates the ST biosynthetic genes, at a transcriptional and post-transcriptional level. The latter regulation is demonstrated by the inability of AflR to function when expressed in a *pkaA* overexpression background. We hypothesize that PkaA post-transcriptionally regulates AflR by phosphorylating some or all of three putative PKA phosphorylation sites in AflR. We are testing this hypothesis by introducing mutations in one, two, or all three of these phosyphorylation sites and asking if any of these AflR mutants function when expressed in a *pkaA* overexpression background. Currently we have been able to construct one of these strains where one PKA phosphorylation site is mutated. In contrast to the inability of a wild type *aflR* allele to induce *stc* gene expression in a *pkaA* overexpression background, expression of the mutant *aflR* allele in the same *pkaA* overexpression background resulted in *stc* gene expression and ST production. These results

suggest that AflR may be a direct target of PkaA and that phosphorylation of AflR negatively regulates its ability to promote *stc* transcription.

374 **Plant cell wall degradation by *Aspergillus nidulans***. Sunita Macwana[1], Anamika Ray[2], Rolf A. Prade[1] and Andrew Mort[2]. [1]Department of Microbiology & Molecular Genetics and [2] Biochemistry & Molecular Biology, Oklahoma State University, Stillwater OK 74078

Little is known about the plant cell wall degrading enzymes produced by *A. nidulans* while growing on plant cell walls. Our study is based on a molecular negative screening method, specifically directed to recover cDNA clones from ALL transcripts *A. nidulans* induces when shifted from growth on glucose to a range of cell wall polysaccharides including pectins, cellulose and xylan. cDNAs prepared from mRNA of tissues grown in glucose were labeled and used to probe a cDNA-plasmid library made from mRNAs extracted from tissues grown on cell wall polysaccharides. Transcripts present in both, the probe and the plasmid library, appear as positives whereas transcripts expressed only in the plasmid library are the negatives (not labeled). A two-staged screening method was devised to allow the survey of a large number of clones for identification of putative negatives in the initial stage followed by reliable confirmation of clones that are not expressed or present in low abundance in the probe originating condition. A statistically significant collection of negatives has been isolated and sequenced for a digital gene expression profiling and functional annotation analysis. Genes recognized through this method, are the ones upregulated as a result of the physiological shift (change in carbon source). Thus, the suggested approach is comprehensive because one can identify whole gene sets activated by a special physiological condition.

375 **Scanning mutagenesis reveals that arginine-specific regulation of *Neurospora crassa arg-2* is dependent on specific residues of the peptide encoded by an upstream open reading frame in its transcript**. Christina C. Spevak, Peng Fang, and Matthew S. Sachs, Oregon Graduate Institute of Science and Technology, Beaverton, OR 97006-8921

The 5' regions of eukaryotic mRNAs often contain upstream open reading frames (uORFs) that are important in cell growth and development. The *Neurospora crassa arg-2* uORF encodes an evolutionarily conserved 24 amino acid peptide, called the Arginine Attenuator Peptide (AAP), which inhibits translation of ARG-2 in a high concentration of arginine by causing ribosomes to stall at the uORF stop codon, blocking ribosomes from reaching the ARG-2 initiation codon. Alanine- and proline-scanning of amino acids 6-24 of the AAP resulted in the identification of the amino acids that were necessary for this regulation in an *N. crassa* cell-free translation system. AAP residues D12, Y13, L14, and W19 were critical for regulation. For residues S6, T9, D16, H17, R20, A21 and A24, regulation was either diminished or lost after mutation to alanine or proline; unlike the case for the wild-type control, regulation was observed to increase as the concentration of arginine increased for these mutants. Mutation of residues V7, F8, L22, and N23 did not affect regulation. The specificity of negative regulation with the wild-type AAP in response to L-arginine was tested using the analogs, agmatine, D-arginine, L-argininamide, L-arginine methyl ester, citrulline, and L-monomethyl arginine. The results indicate that regulation is stereospecific and requires the amino- and guanidino- groups of arginine.

376 **Characterization of the *uvsI* gene encoding an error-prone DNA polymerase and its null mutant in *Aspergillus nidulans*.** Kyu-Yong Han[1], Young-Kug Jang[2], Suhn-Kee Chae[2], and Dong-Min Han[1]. [1]Wonkwang University, Division of Life Science, Iksan, Chonbuk, Korea, and [2]Paichai University, Division of Life Science, Taejon, Korea

Polymerase is an error-prone DNA polymerase, consisting of at least two subunits: REV3 of the catalytic component and REV7 of unknown function in yeast. This complex is responsible for translesion DNA synthesis in consequence often to generate mutations. In *Aspergillus nidulans*, the *uvsI* gene has been cloned and the 9.1 kb DNA fragment containing the *uvsI* ORF was sequenced. UVSI had well conserved the hexapetide motifs of DNA polymerases in the C-terminus and showed the highest amino acid similarity to REV3. In addition, protein interaction of UVSI with yeast REV7 was demonstrated using the yeast two-hybrid system, indicating that UVSI is a functional homolog of REV3. About 5.3 kb *uvsI* transcript was detected in northern analysis and its amount was increased in responses to UV and MMS treatments. A null mutant having the deletion of the entire *uvsI* ORF was constructed. The phenotype of *uvsI* mutant was much the same as that reported for *uvsI501* mutant by showing high UV-sensitivity and reductions of spontaneous and UV-induced reversions of certain mutant alleles. No growth defect was exhibited in

uvsI. Among genes responsible for mutation induction in *Aspergillus nidulans*, *uvsC* (a *recA* and *RAD51* homolog), *uvsJ* (a *RAD6* homolog), and *uvsI*, synergistic interaction in terms of MMS-sensitivity was observed between *uvsI* and *uvsC*. *uvsJ* and *uvsC* showed synthetic lethality. Test for epistatic relationship between *uvsI* and *uvsJ* is currently undertaking. Sequencing of an upstream region of the *uvsI* gene revealed another ORF of 1,401 bp without a putative intron in opposite direction. This ORF starts 365 bp upstream from the *uvsI* start codon and encodes a putative polypeptide exhibiting a high amino acid similarity to SLU7 involving in the second step of pre-mRNA splicing in *S. cerevisiae*. The amount of 1.5 kb transcript from the SLU7 homolog of *Aspergillus* increased after UV irradiation.

377 **Effects of *uvsJ*, a *rad6* homolog, on mutagenesis in *Aspergillus nidulans***. Young-Kug Jang, Etta Kafer[1], and Suhn-Kee Chae. Research Center for Biomedicinal Resources and Division of Life Science, Paichai University, Taejon, Korea, and [1]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada.

In *Aspergillus nidulans*, lack of mutagen-induced mutations has been observed in mutants of two different epistasis groups, UvsI (*uvsI; REV3* homolog) and UvsC (*uvsC; RAD51* homolog). In yeast, RAD6 protein is indispensable to generate mutations. However, the function of RAD6 is mostly unknown except its ubiquitin conjugation activity. To investigate whether the RAD6-dependent mutation pathway is also operated in *Aspergillus nidulans*, we have been cloned a *Rad6* homolog (*radB*) and characterized its null mutant. Unexpectedly we found that *radB* is an allele of *uvsJ* previously assigned in the UvsF group. We also found that *uvsJ1* was a temperature sensitive mutant showing the same level of mutagen-sensitivity to wild type at the permissive temperature (25°C) but demonstrating high sensitivity to MMS and UV-radiation at 37°C similar to *uvsJ* null mutants. Disruption of *uvsJ* caused a slow-growth phenotype on an agar plate, indicating its requirement on normal growth. Such a phenotype was not seen in *uvsJ1* mutant carrying a single point mutation at 58th amino acid histidine. In contrast to yeast *rad6* mutants, *uvsJ* null as well as *uvsJ1* mutants exhibited increased UV-induced mutation frequencies in the system detecting selenate resistant forward mutations when compared with that for wild type. However, UV- induced reversions of *choA1* and *pabaA1* mutant alleles in *uvsJ* null and *uvsJ1* mutants were not detected. In using yeast two- hybrid assay system, UVSJ-UVSH (RAD18 homolog) interaction has been demonstrated, while UVSJ1 mutant protein exhibited reduced affinity to UVSH at 37°C in yeast *in vivo*. Forced over-expression of UVSJ-[C88A], an E2 enzyme active-site mutant protein, in *uvsJ*$^+$ background resulted in the change of colony growth, indicating a dominant-negative effect of the mutant protein.

378 **A RAD52 homolog in *Aspergillus nidulans* as an interactor of UVSC and characterization of its null mutants.** Seung-Hyen Ka, Nam-Sihk Lee, Etta Kafer[1], and Suhn-Kee Chae. Research Center for Biomedicinal Resources and Division of Life Science, Paichai University, Taejon, Korea, and [1]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada.

In *A. nidulans*, differing from *rad51* of yeast, involvement of *uvsC*, a *Rad51* homolog, on mutagenesis has been clarified in various test systems. As an interactor protein with UVSC, we have been isolated a Rad52 homolog using the yeast two-hybrid screening system. Genomic DNA clone of the *RAD52* homolog, named as *RadC* was isolated from a chromosome III specific library and the 10 kb *Pst*I fragment was subcloned. Sequence determination of genomic and cDNA of *radC* revealed an ORF of 2,001 bp interrupted by 4 introns, encoding a polypeptide of 582 amino acids. The deduced amino acids has 54% and 45% sequence similarity to MUS- 11 of *N. crassa* and RAD22 of *S. pombe*, respectively. The C- terminal extension of RADC was observed compared to RAD52 of yeast and a human RAD52 homolog. About 4 kb *radC* transcript was detected in northern analysis and its amount was increased in responses to MMS treatments. *RadC* null mutant was generated by targeted replacement and confirmed by Southern analysis. Null mutants of *RadC* exhibited high MMS-sensitivity and enhancement of spontaneous and UV-induced selenate resistant mutation frequencies. Similarly to *uvsC*, *radC* was not sensitive to UV at dormant conidia stage, but the sensitivity to UV-radiation increased during germination. Epistatic interaction was detected in double disruption mutants of *uvsC* with *radC*. RADC-UVSC interaction was confirmed in both in vivo using the yeast two-hybrid assay and in vitro with a GST-pull down assay. The C-terminus of RADC not well conserved among RAD52 homologs was responsible to interaction with UVSC. In fact, yeast RAD51 failed to interact with RADC *in vitro*, while human hsRAD51 did.

379 **Molecular Cloning and Characterization of an *aga* Mutant from *Neurospora crassa.*** Gloria E. Turner, Sean Curran & Richard Weiss. UCLA, Chemistry & Biochemistr, Los Angeles, CA, USA

The complex *aga* locus encodes 2 forms of the catabolic enzyme arginase (E. C. 3.5.3.1.). Tandem promoters regulate the production of a 1.7-kb and a 1.4-kb transcript, which are translated into 41-kDa and 36-kDa products. These proteins differ at their amino terminus but carry out identical reactions, the hydrolysis of arginine to ornithine and urea. All vertebrates have 2 arginase proteins, which are encoded by separate genes. The liver arginase, A1 is found in the cytoplasm and the extrahepatic enzyme, A2 is found in the mitochondrion. Interestingly both *N. crassa* forms are localized to the cytoplasm. The 36-kDa form is expressed under all growth conditions tested, whereas, the expression of the 41-kDa form is activated by arginine. Both forms are expressed under nitrogen limitation. Recent results have shown that conidia store both forms of the protein and that expression is regulated both temporally and by a number of arginine related metabolites. We have cloned and characterized an *aga* mutant allele UM 913. This mutant lacks arginase enzymatic activity and makes no detectable proteins and a slightly detectable transcript. We have used this mutant as a recipient strain for gene targeting of altered *aga* allowing us to test the outcome of having a single arginase. To fully interpret the results we obtained from our single arginase transformant analysis; UM 913 arginase was cloned and sequenced. We have determined that a frame shift in the first third of the protein leads to an early termination of both proteins. We are presently investigating mechanisms for the reduced transcript levels. It is well documented in other organisms that premature translation termination can reduce mRNA stability.

380 **Salt-stress, *hogA*-dependent control of hyphal growth, branching and septum formation in *Aspergillus nidulans.*** Kap-Hoon Han and Rolf A. Prade. Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater OK 74078 USA

In yeast the HOG pathway senses extracellular salt concentrations and regulates the expression of stress-response-genes that overcome the osmotic imbalance. Based on molecular data derived from yeast, key genetic components that assemble the HOG-pathway were identified and isolated from the *A. nidulans* EST collection. To determine whether the *A. nidulans* HOG-pathway functions similarly as in yeast, the *A. nidulans hogA* gene, yeast hog1 homolog, was isolated and fully characterized. HogA shares 74% identity of its amino acid sequence with *Saccharomyces cerevisiae* HOG1. Northern analysis clearly showed that *hogA* is strongly induced after osmotic shock. Thus, abundance of *hogA* transcript is tightly associated with extracellular salt levels. A *hogA* deletion mutant shows no drastic morphological alteration when cultivated at 37C and normal (low) salt. When grown in the presence of high concentrations of salt, several morphological alterations were detected and studied in detail: First, polar hyphal extension rates are retarded by as much as 60% and, second, hyphal tip morphology is affected in the mutant. Hyphal tips of the mutant are bulbous and hyper-branched. Fluorescence microscopy, employing DAPI and Calcofluor, showed that nuclei accumulate abnormally at the tip of hyper-branched hypha, suggesting normal mitotic division of nuclei, while hyphal extension is severely restricted. Moreover, septum staining revealed abnormal septation patterns at the terminal portion of vegetative hyphae. No septa were observed at the terminal hyphal portions and abnormal chitin deposition was observed at every branching point, implying that *hogA* mutant attempted to form a septum but failed. Our results indicate that the *hogA*-dependent response towards stress in multicellular organisms, in addition to compensate solute imbalances, also appears to control proper cell expansion and modulate formation of cellular compartments.

381 **Genes involved in sterigmatocystin expression in *Aspergillus nidulans.*** Nanci O. C. R. Dezotti, and Tania M. A. D. Zucchi. Department of Parasitology & Biotechnology Research Center; Institute of Biomedical Sciences; University of Sao Paulo, Av. Prof. Lineu Prestes, 1374, Cidade Universitaria, CEP: 05508- 900, Sao Paulo, Brazil.

The fungus *Aspergillus nidulans* (*Emericella nidulans*) was used as a genetic model to investigate the genes, which are involved in sterigmatocystin production. The focused gene was stc, related to sterigmatocystin (ST) expression, which is an intermediate penultimate product in the aflatoxin biosynthetic pathway. The genetic analyses included studies of the sexual and parasexual cycles. The allelic segregation rates and recombination frequencies between linked and non-linked genetic markers were determined by crossing the strains UT448 to UT196 and to UT184, phenotypically stc, stc+, stc, respectively. Low ST expression (4.0 ppm) in the UT196 strain and in 7.4% of meiotic segregants allowed to map the stc locus at chromosome I, 3.4% distant from riboA1. The diploid UT448 (stc)//UT184 (stc) prepared from both non- producing strains was analyzed via parasexual cycle and 28% of its haploid segregants expressed ST. The results indicated that UT448 bears the stc mutant (or inactivated) allele, and

the UT184, although bearing the stc+ allele, it is only reactivated by a factor (R2+), at chromosome VIII of UT448. In such configuration, the diploid expresses large amounts of sterigmatocystin (40 ppm). Another regulator factor (R1) at the meth-w (II) interval was identified in the UT448 strain. Suppot by FAPESP and CNPq/Brazil

382 **Characterisation of two components of the unfolded protein response pathway from the filamentous fungus *Trichoderma reesei*.** Mari Valkonen[1], Markku Saloheimo[1], Michael Ward[2] and Merja Penttila[1] [1]VTT Biotechnology, P. O. Box 1500, FIN-02044 VTT, Finland [2]Genencor International, Inc., 625 Page Mill Rd., CA 94304-1013, USA

The unfolded protein response (UPR) of yeast has been studied intensively and several components of the pathway have been identified. *HAC1* gene encodes a transcription factor that has been shown to bind a consensus sequence in the promoter region of the UPR-inducible genes and activate their transcription. Unconventional splicing involving Ire1p activates *HAC1*. Ire1p is a transmembrane protein kinase/RNase that has been located to the ER membrane. The yeast Ptc2p has been shown to negatively regulate the UPR pathway. We have cloned the *ire1* and *ptc2* genes from the filamentous fungus *Trichoderma reesei* using *Aspergillus nidulans* gene fragments as probes. These were identified in a search with the yeast sequences against *A. nidulans* EST database where cDNA clones were found that were homologous to the yeast genes. The putative *Trichoderma* PTCII protein shows 48% identity to *S. cerevisiae* Ptc2p and 60% identity with a putative PTC2 protein from *Schizosaccharomyces pombe*. The N-terminal domain of *T. reesei* IREI that presumably faces the lumen of the ER has 24% identity and 39% similarity over an area of 377 amino acids with yeast Ire1p. The C-terminal part with the kinase/RNase domains is 42% identical and 59% similar over an area of 490 amino acids to yeast Ire1p. The expression of *T. reesei ire1* and *ptc2* genes has been studied in DTT-treated and control cells. DTT-treatment causes an initial decrease in the expression of the *ire1*, after which there is recovery. The expression of the *ptc2* gene decreases until 90 minutes of DTT-treatment after which there is a slight increase in the expression. The biochemical characterisation of the *T. reesei* IREI and PTCII proteins is being performed.

383 **Induction mechanism of the *Trichoderma reesei* and *Aspergillus nidulans hac1/A* genes involved in the unfolded protein response.** Markku Saloheimo, Mari Valkonen, Mick Ward[1] and Merja Penttila. VTT Biotechnology, P.O Box 1500, 02044 VTT, Finland; [1]Genencor International, 925 Page Mill Road, Palo Alto, CA 94304-1013, USA.

Genes encoding ER chaperones and foldases assisting in protein folding are controlled by the unfolded protein response (UPR) pathway, i.e. they are induced when unfolded proteins accumulate into the ER. The UPR pathway is best known from *Saccharomyces cerevisiae*. The yeast UPR transcription factor binding to the promoters of the target genes is Hac1p. The *HAC1* gene is activated through a unique mRNA splicing event, where the kinase/RNAse Ire1p cleaves the *HAC1* mRNA at the intron borders, and the exons are ligated together by tRNA ligase.

We have addressed the mechanism of UPR pathway induction in two filamentous fungi, *Trichoderma reesei* and *Aspergillus nidulans*. The functional homologs of the yeast *HAC1* have been cloned from these fungi. Binding of the *T. reesei* HACI protein to putative UPR elements of the *pdi1* and *bip1* promoters of this fungus has been demonstrated. The results obtained suggest that the activation of the *hac1/hacA* genes of these filamentous fungi is more complex than in yeast, including two events. These are splicing of an unconventional intron of only 20 bp in length, analogously to the yeast *HAC1* gene, and truncation of the mRNA at the 5' end, which leaves out an upstream open reading frame (uORF). It it has been shown that both the 5' flanking region and the 20 bp intron reduce the ability of *T. reesei hac1* to complement the yeast *HAC1* disruption. Evidence is also shown that the uORF reduces the formation of the HACI protein in yeast.

384 **A molecular dissection of MAP kinase cascades regulating mating and haploid fruiting in *Cryptococcus neoformans*.** Robert C. Davidson and Joseph Heitman. Duke University, Genetics, Durham, NC, USA

Recent findings reveal that a pheromone induced mating pathway operates in the human fungal pathogen *Cryptococcus neoformans*. To dissect the pheromone response pathway in *C. neoformans*, we first cloned the *CPK1* and *STE7* genes, which encode homologs of the FUS3/KSS1 MAP kinases and the MEK kinase of the *S. cerevisiae* pheromone response pathway. In contrast to those genes involved in mating or haploid fruiting previously identified

that are mating type specific, *CPK1* and *STE7* are present in both MATa and MATalpha cells. We used biolistic transfomation to disrupt the *CPK1* and *STE7* genes in *C. neoformans* and show that both are required for mating in both mating types. In addition, the serotype D MATalpha *cpk1* and *ste7* mutant strains are unable to undergo a mating type specific differentiation called haploid fruiting. However we have been unable to show a role for either CPK1 or STE7 in virulence of *C. neoformans*. In order to assemble these elements into a signaling pathway, we employed the use of epistasis analysis.*STE12alpha* a mating type specific homolog of the transcription factor involved in mating and pseudohyphal growth in *S. cerevisiae*, has previously been shown to be required for haploid fruiting and virulence in serotype D MATalpha strains of *C. neoformans*, but is dispensible for mating. Overexpression of *STE12alpha* suppressed the haploid fruiting and mating defects of *cpk1* or *ste7* mutant strains. However, overexpression of CPK1 also suppressed the haploid fruiting defect of a *ste12alpha* mutant. These epistasis results indicate that the STE12alpha transcription factor and the CPK1 MAP Kinase are not acting in a single linear pathway. Two models are consistent with these findings: first another transcription factor exists downstream of CPK1 that is at least partially redundant with STE12alpha. Alternatively, STE12alpha and CPK1 are acting in separate parallel pathways to regulate mating and fruiting. Current studies are underway to test these predictions and assemble the pathways regulating morphogenesis in *C. neoformans*.

385 **Nitrate is necessary but not sufficient for binding of NirA, a pathway-specific activator of *Aspergillus nidulans*.** Frank M. Narendja, Sabine P. Goller, Markus F. Wolschek, and Joseph Strauss. Zentrum für Angewandte Genetik, Universität für Bodenkultur, Muthgasse 18, A-1190 Vienna, Austria

In *Aspergillus nidulans* the structural genes coding for nitrate reductase (niaD) and nitrite reductase (niiA), share a common promotor region of 1200bp. The two genes are divergently transcribed and are under the control of two positively acting transcription factors: NirA, mediating induction and AreA, which is inactivated in the presence of the repressing nitrogen sources ammonia or glutamine (1). We have previously characterized in vitro (2) and in vivo (3,4) the physiologically relevant cis-acting elements for the two synergistically acting DNA-binding proteins. We have further shown that AreA is constitutively bound to a target sequence located within a central cluster of four GATA sites and is directly involved in opening the chromatin structure over the promoter region thus making additional cis-acting binding sites accessible. Here we show that in vivo binding of the NirA transactivator to the central asymmetric recognition sequence is dependent on nitrate induction but also on a functional AreA protein. Our data suggest a potential new role for AreA in regulation of a post-transcriptional activation step of nitrate transport as ectopic expression of the transporter can not restore nitrate uptake in an areA loss-of-function mutant. An interesting situation is revealed when nitrate inducer and ammonia as repressing nitrogen source are available to the cells simultaneously. First, for a short period of incubation, ammonia does not preclude nitrate uptake and intracellular nitrate levels remain high. Second, at this stage both NirA and AreA are bound to their cognate targets but despite this 'ready-to-go' situation transcriptional activation does not take place. Possible mechanisms preventing the activation of a pre-set transcriptionally competent complex will be discussed.

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Developmental Biology

386 **Differential gene expression in germinating basidiospores.** Lori G. Baker and Sarah F. Covert. Department of Genetics and School of Forest Resources University of Georgia, Athens, Georgia USA

Cronartium quercuum f.sp. *fusiforme* is the causative agent of the southern pine disease known as fusiform rust. This disease is characterized by woody, spindle-shaped galls, which weaken pine trees and reduce their value. In a pathogenic fungus such as *C. q. fusiforme,* spore germination directly impacts dissemination and the spread of disease. Depending upon their environment, *C. q. fusiforme* basidiospores can germinate either directly or indirectly. When *C. q. fusiforme* basidiospores are cast upon certain surfaces, such as pine needles or plastic, they germinate directly by sending out a long, thin germ tube. In contrast on other surfaces, such as oak leaves or glass, *C. q. fusiforme* basidiospores germinate indirectly by sending out a very short, wide germ tube that leads to the formation of a secondary basidiospore. This study utilizes subtractive hybridization techniques to compare gene expression patterns between these two germination fates. By identifying genes that are differentially expressed in these distinct germination events, we will expand our understanding of *C. q. fusiforme's* germination, sporulation, and infection processes and perhaps contribute to our general understanding of the same events in other rust/pathogenic fungi.

387 **Interactions between mating-type proteins from the homothallic ascomycete *Sordaria macrospora.*** Jacobsen S, and Pöggeler, S. Lehrstuhl für Allgemeine und Molekulare Botanik, Ruhr-Uni-Bochum D-44780 Bochum, Germany

Mating type genes control sexual development in ascomycetes. Few is know about their function in homothallic species, which are self-fertile and do not need a mating-partner for sexual reproduction. The mating-type proteins are thought to act as transcription regulator proteins and via protein-protein interaction. To elucidate the function of the mating type genes in the homothallic fungus *Sordaria macrospora*, we have tested them in the yeast TWO HYBRID system. In this system the mating-type proteins SMTA-1 and SMTa-1 have domains capable of activating transcription of yeast reporter genes. The TWO HYBRID analysis for mutual heterodimerization as well as for homodimerization revealed the ability of SMTA-1 to interact with SMTa-1, two proteins which are encoded by different mating types in the related heterothallic species *Neurospora crassa*. Moreover, we gained evidence for homodimerization of SMTA-1. The interaction between SMTA-1 and SMTa-1 was specified by interaction experiments with truncated derivatives of SMTA-1. Furthermore we raised antibodies against SMTA-1 and SMTa-1, respectively and are currently underway to confirm the interaction between SMTA-1 and SMTa-1 by means of co-immunoprecipitation. In the light of these findings possible functions of mating-type proteins in the homothallic *S. macrospora* will be discussed.

388 **Pheromone genes of *Sordaria macrospora.*** Pöggeler, S. Lehrstuhl fur Allgemeine und Molekulare Botanik, Ruhr-Uni-Bochum D-44780 Bochum, Germany

In heterothallic fungi an essential step in the mating process is the recognition of mating-partners via mating pheromones. *Sordaria macrospora* is a homothallic ascomycete and as such a homokaryotic ascospore gives rise to hyphae that can complete the sexual life-cycle. Thus in contrast to heterothallic ascomycetes, a mating-partner with an opposite mating-type is dispensable during the sexual development. In order to analyze the involvement of pheromones in cell recognition and mating in a homothallic fungus, two putative pheromone precursor genes, named *ppg1* and *ppg2*, were isolated from *S. macrospora*. The *ppg1* gene is predicted to encode a precursor pheromone that is processed by a Kex2p-like protease to yield a peptide pheromone that is structurally similar to the alpha-factor of the yeast *Saccharomyces cerevisiae*. The *ppg2* gene encodes a 24-amino-acid polypeptide that contains a putative farnesylated and carboxy-methylated C- terminal cysteine residue. Both genes are expressed during the life-cycle of *S. macrospora*. This is the first description of pheromone precursor genes encoded by a homothallic fungus.

Pöggeler, S. (2000) Two pheromone precursor genes are transcriptionally expressed in the homothallic ascomycete *Sordaria macrospora*. Curr Genet 37: 403-411

389 **Signal transduction of light through ndk-1 to the morphogenesis of perithecia in *Neurospora crassa*.** Kohji Hasunuma, Yasunobu Ogura, Yusuke Yoshida, Naoto Yabe. Yokohama City Univ. Kihara Inst. Biol. Res. Yokohama Japan

By use of a crude membrane fraction of mycelial extract of *Neurospora crassa* an *in vitro* system was developed. The irradiation of the extract by blue light increased the phosphorylation of 15 kDa protein, which was purified and identified to be nucleoside diphosphate kinase and designated to be NDK-1. NDK-1 showed i) gamma-phosphate transferring activity, ii) autophosphorylation activity and iii) protein kinase activity phosphorylating myelin basic protein. A mutant *psp* (*p*hosphorylation of *s* mall *p*rotein) with no phosphorylation of NDK-1 was isolated, and the cDNA included *ndk-1*^{Pro72His} mutation. His tagged NDK-1 and His tagged NDK-1Pro72His showed equivalent activity of i) gamma-phosphate transferring activity. However, the mutant protein showed very reduced activity of ii) autophosphorylation and iii) protein kinase activities. The *psp* mutant lacked ability to stimulate light induced polarity of perithecia. However, the transformation of *ndk-1*^{Pro72Hsi} by cDNA and genomic DNA for NDK-1 restored the ability to stimulate the light induced porality of perithecia. Thus *psp* was identified to be *ndk-1* ^{Pro72His}.

390 **Transcription of the *Achlya ambisexualis* hsp90 gene is regulated by the steroid hormone antheridiol and the encoded Hsp90 protein is a component of specialized heteromeric protein complexes associated (in vertebrates) with steroid receptors.** Shelley A. Brunt and Julie C. Silver. Department of Molecular and Medical Genetics and Division of Life Sciences, University of Toronto at Scarborough, Toronto, Ontario, Canada.

In the oomycete *Achlya ambisexualis* , hyphae of the male strain undergo sexual differentiation upon exposure to the steroid hormone antheridiol, which is produced and secreted by the female strain. Northern hybridization established that the level of hsp90 transcripts, as well as the level of transcripts for several other chaperones (hsp70, hsp60), increased in hormone-treated cells. Nuclear run-on studies established that these increases resulted from the increased transcription of these genes in the antheridiol-treated mycelia. Ongoing studies are addressing the identification of the regulatory proteins and sequences involved in the expression of the hsp90 during steroid mediated development. Earlier studies demonstrated that antheridiol binds with high affinity to a 9S multiprotein complex from *A. ambisexualis* cytosols. In vertebrates, steroid hormone receptors are found in 9S heteromeric complexes (aporeceptors) that exhibit high affinity ligand (steroid hormone) binding. The vertebrate aporeceptors complexes contain several distinct proteins, including Hsp90, Hsp70, p60 (Sti 1), immunophilins (e.g. FKBP51) and others. Hsp90-containing heteromeric complexes isolated from *Achlya*, where shown to contain Hsp90, Hsp70, 61 kDa and 64 kDa proteins, a 56 kDa protein related to the immunophilin FKBP51, as well as several other proteins similar to those observed in vertebrate steroid receptor complexes. Although the antheridiol receptor has not been isolated, the ability of antheridiol to regulate gene transcription and the presence of aporeceptor-like complexes in the mycelium suggest that the antheridiol receptor has functional (and perhaps structural) similarity to members of the steroid hormone receptor superfamily. (Supported by grants to J.C.S. from NSERC Canada)

391 **A member of the p450 superfamily is induced during steroid mediated sexual development in the oomycete *Achlya ambisexualis***. Shelley A. Brunt, Thilaka Sritharan, Evangalia Tomai, Paul Milman, Chai Chen and Julie. C. Silver. Department of Medical Genetics and Microbiology and Division of Life Sciences, University of Toronto at Scarborough. Toronto, Ontario, Canada, M1C1A4.

Sexual differentiation in male and female strains of *Achlya ambisexualis* is controlled by the steroid hormones antheridiol and oogoniol. The *Achlya* steroid hormone antheridiol induces antheridial branching in male strains. These specialized branches subsequently differentiate into antheridia (male gametangia). Coincident with antheridium development male strains synthesize and secrete the steroid oogoniol which mediates the development of oogonia (female gametangia) in female strains. Differential hybridization screens using cDNAs from antheridiol-treated and vegetative hyphae of the male strain E87 identified a hormone-inducible cDNA that was not expressed in vegetative mycelia. This cDNA was found to encode a member of the p450 superfamily. The rapid induction of p450 transcripts requires no new protein synthesis and is therefore a primary response to antheridiol. The deduced amino acid sequence of the mature protein encoded by this cDNA has a predicted molecular weight of 59446 and a pI of 8.52. Analyses of the deduced amino acid sequence suggest that the protein encodes a soluble mitochondrial

protein, i.e., is located in the mitochondrial matrix. To our knowledge this is the first p450 superfamily member isolated from an oomycete. A genomic clone containing the promoter has been isolated and is presently being studied to address the regulation of the p450 gene. (Supported by grants to J. C. S. from NSERC Canada)

392 **Cru1, a fizzy-related protein from Ustilago maydis is required to mating.** Tatiana García-Muse, Sonia Castillo, and José Pérez-Martín. Department of Microbial Biotechnology, Centro Nacional de Biotecnología (CSIC), Campus de cantoblanco-UAM, 28049 Madrid SPAIN

We have cloned a gene from *U. maydis* which encodes a protein which belongs to the Frizzy-related family of cell-cycle regulators. Deletion of this gene affects vegetative growth in Ustilago maydis and ectopic expression induces cell-cycle arrest. This cell-cycle regulator is required to arrest cell-cycle before mating takes place in *Ustilago maydis.* A signal emanating from the pheromone receptor which requires the participation of a MAP kinase cascade is involved in the activation of the Cru1 protein. Potential roles in mating regulation of this protein will be discussed.

393 **Crk1, a CDK-like kinase is required for hyphal development in *Ustilago maydis.*** Elia Garrido and José Pérez-Martín. Department of Microbial Biotechnology, Centro Nacional de Biotecnología (CSIC), Campus de Cantoblanco-UAM, 28049 Madrid, SPAIN

Using an oligonucleotide probe-based screen we have cloned several CDK-like proteins from *Ustilago maydis*. One of these genes, crk1, encodes a protein with sequence similarity with the Cdc2 protein from the same organism. Deletion of this gene in *U. maydis* led to a defective hyphal growth in nitrogen-limited medium. Ectopic expression induces hyphal growth in rich medium. Our preliminary data suggest that Crk1 is negatively regulated by the cAMP/PKA pathway, which represses filamentous growth in *Ustilago maydis*. Potential relations between this pathway and filamentation will be discussed.

394 **The *Cladosporium* hydrophobin *HCf-1* is required for efficient water-mediated spore dispersal.** P D Spanu and J Whiteford. ICSTM, London, UK

The tomato pathogen *Cladosporium fulvum* has at least six hydrophobins (*HCf-1* to *6*). Four of these are Class I and two are Class II hydrophobins. The expression of these genes is differentially regulated and we believe they have different functions. In order to investigate hydrophobin function we are creating hydrophobin gene deletion mutants. So far we have obtained delta*HCf-1*, delta*HCf-2* and delta*HCf-6* single knockouts and a delta*HCf-1/2* double knockout. We can also down-regulate hydrophobin expression by homology-dependent gene silencing. The degree of silencing varies in different transformants and we have selected a highly silenced strain C11. In C11 all Class I hydrophobins are down-regulated. We believe this is due to expression of antisense *HCf-1* RNA; *HCf-5* is unaffected and *HCf-6* expression is higher than in wild type. Growth and sporulation of the knockout mutants analysed so far does not differ from the wild type isolates. On the other hand sporulation of C11 is both delayed and reduced and germination rate of C11 spores is lower. The deletion of the *HCf-1* hydrophobin decreases the hydrophobicity of mycelium and spores in comparison to the other strains. Of particular note is the reduced ability of the spores lacking *HCf-1* to be transported on the water/air interface of water droplets. We therefore propose that one of the main roles of *HCf- 1* is to aid water-mediated spore dispersal.

395 **The LIS1 homolog NUDF of *Aspergillus nidulans* specifically interacts with subunits of dynein, dynactin and microtubles.** Bernd Hoffmann, N. Ronald Morris. Univertity of Medicine and Dentistry of New Jersey, Pharmacology, Piscataway, New Jersey, USA

The human disease lissencephalie or Miller-Dieker syndrome is caused by haploinsufficiency of the *Lis1* gene. This disorder results in drastic changes during brain development finally ending in a smooth brain surface with a strongly reduced amount of neuronal cells. This defect seems to be reasoned by a reduced neuronal motility to the brain surface during embryogenesis because of an affected dynein activity in lis1 mutant cells. We used the filamentous fungus *Aspergillus nidulans* as model organism to study the putative interaction between the Lis1 homolog protein NUDF and its binding protein NUDE with all known dynein, dynactin and microtuble subunits of filamentous fungi. Both proteins interact specifically with subunits of these multiprotein complexes in two-hybrid and coimmuno-praecipitation experiments. While NUDF is able to interact with the intermediate chain of dynein and alpha- or

gamma-tubulin, NUDE forms complexes with dynein light and intermediate chain, the actin related protein NUDK of dynactin and, as NUDF, with alpha- and gamma-tubulin. Point mutations in the dimerization domain of NUDF destroy the ability to interact with the intermediate chain and alpha- but not gamma-tubulin. For all observed interactions of NUDE the N-terminus is sufficient. Posttranslational modifications putatively in this area lead to two NUDE subfractions of which the unmodified form has a high affinity to alpha-tubulin while the modification leads to a drastic change in affinity from alpha- to gamma-tubulin. These data argue for a direct protein-protein interaction between the LIS1 homolog protein NUDF of *A. nidulans* and subunits of dynein/dynactin and for a functional involvement of the protein complex NUDE/NUDF in mitosis via gamma-tubulin binding.

396 **Meiosis and ascospore development in nonlinear asci of *Neurospora pannonica*.** Namboori B. Raju, Stanford University.

Neurospora pannonica is homothallic, with eight-spored asci. Immature asci are usually swollen and noncylindrical while the mature asci are narrow and cylindrical. The two meiotic divisions resemble those of other *Neurospora* species. However, the orientation of third-division mitotic spindles and the distribution of nuclei in the swollen asci are irregular. Ascospores are arranged irregularly at first, but as the ascospores enlarge and mature the asci gradually become cylindrical, with the ascospores aligned in single file. The asci cannot be considered ordered tetrads, because ascospore order does not reflect the assortment of chromosomes at the first and second meiotic divisions. Contrary to the original species description, ascospores require heat shock for germination and hyphae are sent out at both ends of germinating ascospores. Supported by MCB-9728675 from the National Science Foundation.

397 **The genetic basis of abnormal ascospore morphology in *Neurospora*.** Namboori B. Raju[1] and Anna Geng Burk[2]. [1]Stanford University. [2]Cornell Univeristy.

A recessive ascospore mutant, named *bud*, was isolated from a wild- collected, multi-component, heterokaryotic strain of *Neurospora tetrasperma*. When *bud* is homozygous, meiosis is apparently normal but postmeiotic events are abnormal. Orientation of spindles at the postmeiotic mitosis is often abnormal and this results in failed pairwise association of nuclei and their irregular distribution along the length of the ascus prior to spore delimitation. Consequently, many asci cut out more than four ascospores; some contain no nuclei while others contain more than two nuclei. The most dramatic effect of *bud* is on ascospore delimitation itself. Many ascospores are irregularly shaped and are often interconnected, because of incomplete spore delimitation. Ascospores also show one or two lobes or bud-like extensions of varying sizes. Over 90% of ascospores from *bud* X *bud* remain white or tan and are inviable. The interaction of *bud* with a dominant *Eight spore* mutant (*E*) was examined in both heterozygous and homozygous crosses. When both are heterozygous, *bud* has no effect on ascospore delimitation or on the phenotype of *E* because *bud* is recessive, and many asci produce 5 to 8 ascospores just as in *E* X *E*. And when *bud* is homozygous and *E* is heterozygous, ascospore delimitation is less affected than when *E* is absent. Moreover, when both *bud* and *E* are homozygous, the effect on ascospore development is less extreme than when either mutant is homozygous singly. Supported by MCB-9728675 from the National Science Foundation.

398 **Characterization of *ras* mutant alleles from *Coprinus cinereus* homokaryon AmutBmut.** Alan P. F. Bottoli, Robert P. Boulianne, Markus Aebi and Ursula Kües. ETH Zurich, Institute for Microbiology, Schmelzbergstr. 7, CH-8092 Zurich, Switzerland

Using the *ras* gene of *C. cinereus* homokaryon AmutBmut, we produced mutant alleles for a dominant activated Ras protein (Ras-val19) and a dominant inhibitory Ras protein (Ras-asn24). Both ras alleles impaired mycelial growth of transformants of homokaryon AmutBmut on selective minimal medium. This failure to grow was due to loss of the co-transformed selection marker, together with the mutant *ras* gene copies. In the two monokaryons PG78 and 218, a specific growth phenotype was identified only for Ras-val19. Ras- val19 transformants of monokaryon PG78 have a reduced growth rate and show enhanced invasive growth, whereas Ras-val19 transformants of monokaryon 218 exhibit a disoriented pattern of hyphal growth with a general increase in branching. The aerial mycelium has a fleecy appearance with regularly distributed regions of highly proliferating hyphae. These areas are different from the small, more compact primary hyphal knots, structures of local intense branching that give rise to sclerotia (multicellular resting structures). In fact, formation of primary hyphal knots, the initiating process of fruiting body development as well as sclerotia development, was found to be repressed in Ras-val19 transformants on complete medium.

399 **An essential gene for fruiting body initiation in the basidiomycete *Coprinus cinereus* is homologous to bacterial cyclopropane fatty acid synthase genes.** Yi Liu, Sabine Loos, Markus Aebi and Ursula Kues. Institute of Microbiology, ETH Zurich, Schmelzbergstrasse 7, CH-8902 Zurich, Switzerland

Homokaryon AmutBmut is a specific strain of the basidiomycete *Coprinus cinereus* that, due to mutations in the mating-type loci, produces fruiting bodies without prior mating to another strain. The homokaryon has therefore been used for creating mutants in fruiting body development. Early stages of fruiting body development include the dark-dependent formation of primary hyphal knots and the light-induced transition from primary hyphal knots to the more compact secondary hyphal knots (fruiting body initials). UV mutant 6-031 forms primary hyphal knots but development arrests at the transition stage. Genetic analysis indicated that this phenotype is caused by a single recessive defect (*skn1*). Using a SIB-selection transformation procedure, a cosmid was isolated from a genomic DNA library that complemented the defect. The responsible gene (referred as *cfs1*) on this cosmid encodes a protein product highly similar to cyclopropane fatty acid synthases, a class of enzymes so far characterized only in prokaryotes. The *cfs1* allele of mutant 6- 031 carries a T to G transversion, leading to an amino acid substitution (Y441D) in a domain suggested to be involved in the catalytic function. The mutant allele of *cfs1* was unable to complement the fruiting deficiency in strain 6-031, indicating that this gene is essential for fruiting body initiation in *C. cinereus*.

400 **The MAP kinase gene *MAF1* is essential for infection structure formation of *Colletotrichum lagenarium*.** Kaihei Kojima, Taisei Kikuchi, Yoshitaka Takano, and Tetsuro Okuno. Kyoto University, Graduate School of Agriculture, Kyoto, Japan.

Colletotrichum lagenarium, the causal agent of cucumber anthracnose disease, invades the host plant using a specialized infection structure called appressorium. It has been demonstrated that the MAP kinase (MAPK) gene *CMK1* , showing homology to the *FUS3/KSS1* MAPK genes of *Saccharomyces cerevisiae*, is required for diverse aspects of fungal pathogenesis including appressorium formation in *C. lagenarium*. Here we report that the *SLT2* type MAPK gene *MAF1* is essential for appressorium differentiation. *MAF1* encodes a 418 amino acid protein with 91% identity to *Magnaporthe grisea* Mps1 MAPK and 80% identity to *S. cerevisiae* Slt2 MAPK. *maf1* null mutants were created by targeted gene replacement. *maf1* mutants showed normal growth and reduction in conidiation. Pathogenicity of *maf1* mutants was dramatically reduced. On glass and host plant surfaces, conidia of *maf1* mutants germinated but failed to form appressoria. Germ tubes of the mutants continued to elongate without any swollen structures. When conidia of the wild type are incubated on nutrient agar, they elongate germ tubes without appressoria, which is similar to the phenotype of *maf1* mutants on glass. This suggests a possibility that the *MAF1* MAPK pathway is inactive in conidia of the wild type incubated on nutrient agar. The phenotype of *maf1* mutants is distinct from that of *cmk1* mutants that produce swollen structures from germ tubes but fail to complete appressorium formation. These results indicate that the two MAPK genes, *MAF1* and *CMK1*, regulate the distinct steps of appressorium formation independently.

401 **Genetic and molecular analysis of mutations affecting the propagation of two non-conventional genetic elements in *Nectria haematococca*.** Stéphane Graziani, Philippe Silar and Marie-Josée Daboussi. Université Paris Sud, IGM, Orsay FRANCE

One strain of the ascomycete *Nectria haematococca* can display two different morphological modifications caused by two specific cytoplasmic determinants, the 'Anneau' and the 'Secteur'. Once initiated, these modifications spread through anastomoses leading to a growth alteration along its path with a speed (up to 4mm/h) that dictates their form. Recently, it was shown that similar phenomena in fungi are controlled by prion-like proteins. In the light of this discovery, infectious modifications in *Nectria* are now being reconsidered. Both 'Secteur' and 'Anneau' are under the control of nuclear genes. Two kinds of mutations have been detected. Some do prevent expression of both modifications and these map to at least four loci (called *nas*). They are characterized by dense white aerial mycelia and female sterility. The other mutations prevent specifically the formation of either one of the modifications. They are located at a unique locus, locus *S* for the 'Secteur' and locus *A* for the 'Anneau'. These loci are supposed to be direcly involved in the generation of the determinants and thus have to be cloned. The SIB selection failed, but insertional mutagenesis as an alternative cloning strategy allowed the recovery of 10 mutants impaired in the ability to express the 'Secteur'. Sexual crosses indicated that the *S* gene and several *nas* genes are tagged. Presently, a

cosmid that restores the ability to differentiate sectors when introduced in a strain mutated at the S locus is under characterization. Results on the molecular analysis of *nas* mutants will also be presented.

402 **Characterization of differential gene expression in a haploid filamentous mutant of the anther smut, *Microbotryum violaceum*.** Carolyn F Hughes, Malia A Ray, and Michael H Perlin. University of Louisville, Louisville, KY, USA

M. violaceum is dimorphic in that the yeast-like haploid sporidia can mate with other sporidia of opposite mating-type to produce infectious dikaryotic hyphae. This switch is an absolute requirement for infectivity and the remaining stages of development are obligately parasitic. Normally the switch requires a signal from the host plant and this signal does **not** elicit hyphal production from non-mated sporidia. Thus, the appearance of sporidia that grow filamentously raises interesting questions about differences in gene expression among the corresponding cell types: i.e., wildtype sporidia, filamentous mutants, and dikaryotic hyphae. Strain NINE is a haploid sporidial isolate from the wildflower *Silene latifolia*. Prolonged growth of this strain at room temperature allowed the identification of a mutant, NINE-fil, which produces long filaments. Initially, NINE-fil cells appear elongated, but after more than two weeks at room temperature, filamentous growth is obvious on rich media. On low-nitrogen media, the phenotype is more pronounced, with filaments produced after 1-2 days. There is no evidence that NINE-fil is infectious alone, but it is capable of mating. This allowed the demonstration that the filamentous phenotype segregates after passage through the plant. A subtractive cDNA library was constructed using cDNA from the wildtype strain as driver, so as to identify genes up-regulated in the NINE-fil mutant. The results of characterization of these genes as well as comparison with genes known to be up-regulated in dikaryotic hyphae of this organism will be presented.

403 **Surprise, surprise: The smut fungi, *Ustilago maydis* and *Microbotryum violaceum* contain STE20 homologues.** Wei Hong[1], David Smith[1], Scott Gold[2], and Michael H Perlin[1]. [1]University of Louisville, Louisville, KY, USA, [2]University of Georgia, Athens, GA, USA

The mitogen-activated protein kinase (MAPK) pathways are conserved from fungi to humans and have been shown to play important roles in mating and filamentous growth for both yeast and dimorphic fungi, and in infectivity for pathogenic fungi. Upstream of the typical 3-kinase module, *STE20*, encodes a protein kinase of the PAK family that regulates more than one of these cascades in yeasts. We hypothesized that a STE20 homologue would play a similar role in the dimorphic plant pathogens *U. maydis* and *M. violaceum*. Using degenerate PCR primers, portions of the genes for STE20 homologues were amplified from genomic DNA of both fungi and the resulting fragments were sequenced to confirm their identities. The full-length copy of the *U. maydis* gene was obtained from a genomic library and was found to contain a 1782 bp coding region, yielding a predicted protein of 594 amino acids. Two regions of the predicted protein were particularly conserved compared to other STE20 proteins: one in the N-terminal portion, expected to be the binding region for a regulatory factor and the other in the C-terminal portion, corresponding to the kinase catalytic region in the *S. cerevisiae* protein. No intron was found for this gene. The sequence was also obtained for 1299 bp of the coding region for the STE20 homologue from *M. violaceum*, predicted to encode 433 amino acids which also showed homology to the STE20 proteins in GenBank. A knock-out construct was made for the *U. maydis* homologue- to decrease the likelihood of non-homologous recombination, it was used as a PCR product containing only the STE20 gene and a selectable marker inserted within the gene to disrupt its function.

404 **Methylammonium permeases in *Ustilago maydis* and *Microbotryum violaceum* - connection to the cAMP-pathway?** David Smith, Michael H. Perlin. University of Louisville, Louisville, Kentucky, USA

We are investigating the effects of nutrient limitation and its role in regulating fungal dimorphism in plant pathogens. Nutrient stress, such as low ammonium, may be one of many initial signaling events that stimulates mating and hyphal growth. We isolated full-length cDNA clones for ammonium transporters in the corn smut, *U. maydis*, and in the anther smut, *M. violaceum*: *Ump1* and *MEPa*, respectively. These proteins are predicted to have 10-12 transmembrane spanning regions and are believed to have functional similarity to other known ammonium transporters. Their function was tested in a heterologous host. *S. cerevisiae* mutants with no functional ammonium transporters have a severe growth defect on low ammonium media. When transcribed from a yeast expression vector, both *Ump1* and *MEPa* could complement this growth defect. The possible roles of these genes in regulating

dimorphism was studied further by creating knock-outs of the *Ump1* gene in both mating types of *U. maydis*. These strains are unimpaired in their ability to mate and show typical signs of disease. However, after prolonged culture some knock-out strains showed a consistent filamentous phenotype similar to that seen in strains lacking the ability to produce cAMP. The addition of exogenous cAMP to the media restores growth to a budding phenotype. This suggests that *Ump1* regulates the switch to filamentous growth in a cAMP-dependent manner.

405 Withdrawn

406 **Molecular cloning of the *laeA* gene regulating *aflR* expression in *Aspergillus nidulans.*** Jin Woo Bok and Nancy P. Keller. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843-2132

AflR is a C6 zinc cluster protein positively regulating the expression of biosynthetic genes required for sterigmatocystin (ST) production in *A. nidulans* and aflatoxin (AF) in *A. flavus* and *A. parasiticus*. Loss of expression of *aflR* leads to loss of ST/AF production. Here we describe progress in characterizing an *A. nidulans* loss of *aflR* expression (*lae*) mutant. *LaeA1* was complemented by transformation with a trpC library. Subcloning of the cosmid rescued from the *laeA* wild type transformant showed that the complementary DNA fragment contained an ORF of ca. 3.0 kb. This ORF has no homology to any gene in the database. mRNA analysis reveals a 2.3 kb transcript that is developmentally regulated and appears prior to *aflR* expression. Two putative AflR binding sites are found in the promoter of *laeA*.

407 **Morphological mutants of *Neurospora* from nature.** Alka Pandit and A. J. Griffiths. Department of Botany, University of British Columbia, 6270 University Boulevard, Vancouver, B. C., Canada, V6T 1Z4.

In order to understand growth and morphogenesis of fungi *Neurospora* has been extensively used for genetic investigations. Most of the attention has been focussed on mutants derived in the laboratory. Strains from nature have been rarely investigated for their morphological abnormalities. We have screened a local population of *Neurospora* from India for morphological defects. Surprisingly many of these natural strains show distinct morphological defects. Ujjain ûû1 is one such *N. crassa* strain which shows morphological abnormalities like erratic growth rate and defect in branching. Upon crossing this strain with ORS-6a an interesting mutant has been recovered in the progeny (V2-17). This strain (V2-17) has a peculiar growth pattern which is unlike any other known mutant. The main hypha bends down at the tip and the fungus grow forward by the extension of the lateral branch, which again bends at tip after some growth and the pattern is repeated. We are trying to characterise these mutants to determine the number of loci involved and their possible functions. The existence of these morphological mutants in nature raise many interesting questions, like their origin and possibility of being carried in heterokaryons. Although it may appear far fetched the idea that these mutations may be adaptive can not be ruled out. The study of these mutants may help in answering these questions about the natural populations of *Neurospora*.

407 ***Neurospora crassa* cold-sensitive mutants potentially significant for elucidation of hyphal morphogenesis**. Aleksandra Virag, and Anthony J.F. Griffiths. University of British Columbia, Vancouver, Canada.

The goal of this study is to identify a class of cold-sensitive mutants that will be useful for investigating hyphal morphogenesis. Sites of tip growth and branching in *N. crassa* are locations at which complex processes occur. They result in a characteristic dynamic change in the distribution of hyphal components. Among these components are microfilaments organized into a cortical network. When shifted to low temperatures, wild type strains respond with a cold-shock reaction that transiently changes the branching frequency and pattern (Watters *et al.*, 2000). Low temperatures also affect polymerization of F-actin filaments. Because of its presence at all active sites of growth, actin in the form of microfilaments could, directly or indirectly, contribute to tip growth and branching processes both in standard (25 degrees C) and low temperature conditions (7 degrees C). Cold-sensitive mutants may point to the presence of modifications in actin or actin related proteins. However, a wide range of molecular and cellular events not related to actin and microfilaments can also result in sensitivity to cooling. Selection for mutations causing cold sensitivity as a result of microfilament modification was achieved by selecting for mutant strains that in addition to cold sensitivity are resistant to cytochalasin A, or show a different branching behaviour. Three main categories of mutant strains were obtained by UV mutagenesis, and identified. Beside cold-sensitivity, at standard

temperature conditions these strains showed higher branching frequency than wild type, lower branching frequency than wild type, or cytochalasin A-resistance coupled with a transient loss of hyphal orientation upon shift to low temperatures. Characteristics of these strains are described. This work was supported by NSERC.

409 **The role of G-proteins in the regulation of dimorphic switching in *Penicillium marneffei.*** Sophie Zuber, Michael J. Hynes and Alex Andrianopoulos Department of Genetics, University of Melbourne, 3010 Victoria, Australia

The opportunistic human pathogen *P. marneffei* is a dimorphic ascomycete able to switch between two growth forms in a temperature dependent manner. At 25 C, *P. marneffei* exhibits filamentous growth by producing unconstricted branching hyphae, whereas at 37 C it grows as unicellular yeast dividing by fission. We are interested in understanding the mechanisms controlling the dimorphic switch and in particular the temperature sensing mechanism and the signal tranduction pathway it triggers. Heterotrimeric G- proteins control developmental programs in fungi and other organisms acting as signal transducers that couple cell surface receptors to cytoplasmic effector proteins and have been implicated in dimorphic switching in other fungal species such as *Saccharomyces cerevisiae* and *Ustilago maydis.* Therefore, we are interested in determining if heterotrimeric G-proteins are involved in the temperature-dependent dimorphism exhibited by *P. marneffei.* We have cloned by homology genes encoding three G-protein alpha-subunits from *P. marneffei*, designated *gasA, gasB* and *gasC,* representing one of each major subgroup within the family of fungal G-protein alpha-subunits. GasA and GasC are members of subgroup I and III, respectively, which are related to the mammalian $Galpha_i$ and $Galpha_s$ proteins. GasB falls into subgroup II which has no corresponding mammalian counterpart. GasA is highly homologous to the *Aspergillus nidulans* FadA, a key component in the regulation between growth and development. The *gasB* gene shows strongest homology to the *A. nidulans ganA* gene. GasC is closely related to the *A. nidulans* GanB, *Magnaporthe grisea* MagA and the *S. cerevisiae* Gpa2 proteins, the latter playing a crucial role in signalling leading to pseudohyphal growth. Using mutational analysis we have examined the function of these genes by (I) construction of gene-replacement knock-out mutants in *P. marneffei* and (II) generation of dominant activating and dominant interfering alleles producing constitutively active or inactive proteins, respectively. We have introduced these into *P. marneffei* to characterise the role of these signalling components in dimorphic switching, asexual development and growth.

410 **Fruiting body size regulation via a factor which modulates cAMP signal transduction.** Richard H. Gomer, Lei Tang, Robin Ammann, Celine Roisin, and Wany Jang. Howard Hughes Medical Institute and Department of Biochemistry and Cell Biology, MS-140, Rice University, 6100 S. Main Street, Houston, TX 77005-1892

When starved, cells of the primitive fungus *Dictyostelium discoideum* aggregate in dendritic streams which break up into groups of ~2 x 10^4 cells. A secreted 450 kDa protein complex, counting factor (CF), regulates the size of these groups. High levels of CF cause the streams to break into small groups, while cells lacking CF form huge groups, with the resulting fruiting bodies toppling over. The aggregation is mediated by relayed pulses of extracellular cAMP. cAMP activates a receptor which activates G proteins, which activate PI3 kinase. This then modifies plasma membrane lipids, allowing a protein called CRAC to bind to the membrane and activate adenylyl cyclase. The G proteins also cause a transient activation of guanylyl cyclase. We find that CF slowly down-regulates the guanylyl cyclase activity. In contrast, a one-minute exposure of cells to CF increases the cAMP-induced cAMP pulse. This is accomplished by potentiating adenylyl cyclase via a pathway that is downstream of the cAMP receptors/ G protein/ PI3K/ CRAC pathway. The internal pulses of cGMP and cAMP regulate cell motility and the expression of cell-cell adhesion molecules. We find that via this pathway CF modulates adhesion and motility. Low levels of CF decrease motility and increase adhesion, resulting a stream staying intact. High levels of CF increase motility and decrease adhesion, causing the stream to break up. Computer simulations indicate that as a general principle, a secreted signal regulating motility and adhesion can regulate group size. Similar pathways may thus regulate tissue size in other systems.

411 **Cat-1, an endurable large catalase of *Neurospora crassa* conidia.** Díaz, A., Lledías, F., Michán, S., Rangel, P., Montes de Oca, Y. and Hansberg, W. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ap. postal 70-242, México 04510 D.F.

Cat-1 constituted 0.6% of total protein in conidia, where it was localized mainly at the cell wall. Cat-1 activity decreased during germination and was substituted by Cat-3 during growth. Activity increased with conidiation, heat shock, light and singlet oxygen. Cat-1 is a homotetramer of 88 kDa subunits containing each a 679 Da chlorin instead of protoheme IX. The tetramer pI was 5.25. The protein had 30 hexoses per subunit. Cat-1 crystal structure showed a 'flavodoxin-like' domain at the C-terminal and the N-terminal arm of a monomer slipping through of the 'wrapping domain' of the Q-related subunit and vice versa. The enzyme was stable in organic solvents, high salt or SDS concentrations. Urea 9 M or 3.5 M guanidine-HCl partially inhibited the enzyme, but activity was recovered upon dialysis. A *K*m of 22 mM was obtained with less than 100 mM H2O2 and of 233 mM at molar H2O2 concentrations. It exhibited no pH optimum and was inactivated by 3-amino-triazole, azide and hydroxylamine. KCN was a competitive inhibitor with a *K*i of 0.76 mM. Cat-1 is modified by singlet oxygen giving rise to more acidic active conformers. Modification site is at the chlorine. Compared to the non-oxidized conformer, the fully oxidized enzyme had a lower *K*m at molar concentration of H2O2, was more sensitive to KCN, and less stable at an acidic pH and in the presence of guanidine-HCl. The gene has one intron, codifies for a 737 amino acid polypeptide. It is 64% similar to *Aspergillus nidulans* CatA, 66% to *A. fumigatus* CatA and 53% to *N. crassa* Cat-3. Accumulation of the mRNA was observed at the stationary growth phase and late during conidiation.

412 **Cat-3, a light-inducible catalase in *Neurospora crassa* mycelia.** Michán, S., Díaz, A., Lledías, F., Beltrán, M., Montes de Oca, Y., Martínez, C. and Hansberg, W. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ap. postal 70-242, México 04510 D.F.

Cat-3 activity increased during the growing and pre-stationary phase, adhesion of hyphae and growth of aerial hyphae. The enzyme was localized at the cell wall but also in mitochondria and the cytosol. Cat-3 activity increased with depletion of the carbon source, paraquat, light and singlet oxygen. Cat-3 is a homotetramer of 79 kDa subunits, probably containing a chlorin instead of protoheme IX. The tetramer pI is 4.80. The protein was slightly glycosylated. The enzyme was stable in organic solvents, high salt or SDS concentrations. Cat-3 was resistant to molar concentrations of H2O2. A *K*m of 74 mM was obtained with less than 100 mM H2O2 and of 209 mM at molar H2O2 concentrations. It exhibited no pH optimum and was inactivated by 3-amino-triazole, azide and hydroxylamine. KCN was a competitive inhibitor with I_{50} of 60 microM. Cat-3 is also modified by singlet oxygen giving rise to more acidic active conformers. The gene has two introns, codifies for a 719 amino acid polypeptide. It is 67% similar to *Claviceps* Cat1, 65% to *Aspergillus nidulans* CatB and 64% to *A. fumigatus* CatB and 53% to *N. crassa* Cat-1. Accumulation of the mRNA was observed at the pre-stationary growth phase. Accumulation of the mRNA by light was dependent on both 'white collar' transcription factors. Initiation sites and the polyadenilation site were determined. Paraquat regulated *cat-3* expression at the transcriptional level.

413 **Neurospora crassa catalase-2 is a developmentally regulated catalase- preoxidase.** Peraza, L., Montes de Oca, Y., Michán, S., and Hansberg, W. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ap. postal 70-242, México 04510 D.F.

Catalase-2 (Cat-2) activity was detected in mycelia at the stationary growing phase or when deprived of the carbon source. Activity increased in aerial hyphae and in conidia. High levels of Cat-2 activity were found in heat shocked or H2O2- treated mycelia or when conidiation takes place in a medium with ethanol, methanol, uric acid, acetate or fatty acids as sole carbon source. Immunodetection and cell fractionation experiments indicated that the enzyme is located partially in the cell wall. Cat-2 was purified to homogeneity. The enzyme is a homodimer of 89 kDa subunits. Absorption spectrum and activity inhibition with cyanide indicates that it is a heme protein. Cat-2 has both catalase and peroxidase activities. *O*-dianisidine, diaminobenzidine, guaiacol and ascorbate are accepted as electron donors but not NADH, NADPH, glutathione or cytochrome *c*. Cat-2 has a *K*m of 9.02 mM and a high catalytic efficiency. Optimum pH for catalase and peroxidase activity is 6.3 and 4.7 respectively. The kinetic constants, pH optimum, and a low inhibition with 3-amino-triazole are features similar to other catalase-peroxidases. The sequence of an internal polypeptide confirmed that Cat-2 is a catalase-peroxidase. The sequence obtained coincided with part of the predicted peptide from the cDNA sequence NM6H12 (GenBank Acc: AA901970 & AA901741) which has high similarity with bacterial catalase-peroxidases. Cat-2 mRNA accumulation was observed in the aerial hyphae 18 h after induction of the conidiation process.

414 **het-s: an heterokaryon incompatibility gene encoding a fungal prion.** Coustou V, Maddelein ML, Dos Reis S, Schaeffer J, Saupe SJ and Begueret J. Laboratoire de Genetique Moleculaire des Champignons, IBGC UMR, 5095 CNRS Universit de Bordeaux 2, 1 rue Camille St Sains,33077 Bordeaux cedex, France

Prions are proteinaceous infectious particles that cause spongiform encephalopathies in mammals (1). Proteins that behave as prions (infectious proteins) have been described in yeast and in the filamentous fungus Podospora anserina. They correspond to the Ure2p, Sup35 (2) and HET-s proteins (3, 4). They have been termed prion proteins because they exist as two alternate states: a soluble form and a transmissible (infectious) and aggregated form. The het-s gene is one of the heterokaryon incompatibility genes of P. anserina. Strains of the het-s genotype can exist as two alternate incompatibility phenotypes : the neutral [Het-s*] and the active [Het-s] phenotype. A [Het-s*] strain is converted to the [Het-s] phenotype upon contact with a [Het-s] strain. Using GFP fusion proteins, we show that the transition from the [Het-s*] to the [Het-s] state (prion state) leads to in vivo aggregation of the protein. Purified his-tagged recombinant HET-s protein which is initially soluble and monomeric, forms aggregates after several days at 4 C. These aggregates trigger the aggregation of fresh soluble protein. They bind congo red and show birefringence under polarised light, a property of amyloids. Characterization of these aggregates by electron microscopy indicated that they are composed of ordered unbranched fibrils of about 25 nm in width and up to several micrometers in length. (1) Prusiner (1998) Proc. Natl. Acad. Sci. USA 95: 13363 (2) Wickner et al. (1999) Microbiol. Mol. Biol. Rev. 63: 844 (3) Coustou et al (1997) Proc. Natl. Acad. Sci. USA 94: 9773 (4) Coustou et al (1999) Genetics 153: 1629

415 **Regulation of spore germination in *Colletotrichum graminicola* by environmental signals.** L. Vaillancourt [1], J. Chaky [2], and E. Nuckles[1]. [1]Department of Plant Pathology, University of Kentucky, Lexington KY [2] Department of Plant Pathology, University of Nebraska, Lincoln NE

Spore germination is not only an interesting problem in developmental and cell biology; it represents a potentially important target for novel antifungal therapies. However, the molecular regulation of spore germination in filamentous fungi is poorly understood, even in the better-studied model systems like *Neurospora* and *Aspergillus*. In our laboratory, we have initiated a study to elucidate the molecular mechanisms that control germination in the plant-pathogenic fungus *Colletotrichum graminicola*. Spore germination in *C. graminicola* is environmentally responsive. Breaking of spore dormancy occurs only when specific environmental cues are received. Subsequent germ tube development is also controlled by environmental cues, particularly by physical aspects of the substrate. We have begun dissecting the response of the spore and the germling to its environment, using a four-fold approach. First, we are characterizing and quantifying the spore germination process as it occurs in various artificial environments. Second, we are using pharmacological inhibitors to indicate which molecular signaling pathways might be involved. Third, we are creating random insertional mutants and screening for those that are deficient in spore germination. And fourth, we are cloning *C. graminicola* homologues of genes that are known or suspected to play a role in spore germination and development in other fungi. The roles of these genes in *C. graminicola* will be determined by knockout experiments. Our progress in using these approaches to elucidate molecular mechanisms of spore germination and subsequent development will be presented.

416 **Demonstration of circadian rhythms in *Aspergillus*** Andrew Greene*[1,2], Nancy Keller[1, 3], and Deborah Bell-Pedersen[1, 2]. [1]Program For the Biology of Filamentous Fungi, [2]Department of Biology, [3]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843

Endogenous circadian oscillators have been described in a variety of organisms ranging in complexity from cyanobacteria to humans. The filamentous fungi *Neurospora*and *Aspergillus*are ideal organisms in which to study the molecular basis of the circadian clock because of their well-developed genetics and ease of manipulation in the laboratory. Furthermore, in *Neurospora*, the circadian clock controls asexual spore production, providing an easy means to assay rhythmicity. Most studies of circadian rhythms in fungi have been carried out in *Neurospora*; no rhythms in *Aspergillus* have been reported. Unlike *Neurospora*, *Aspergillus* is of extreme agricultural importance because of its production of the potent carcinogen aflatoxin, which causes billions of dollars of crop losses each year. Previous studies have demonstrated that development and toxin production are closely associated. Therefore, our goals are to develop *Aspergillus* as a model system for studies of circadian rhythms and to investigate the link between the circadian clock, development and toxin production. As first steps towards these goals, we have established the presence of a circadian rhythm in sclerotial production in *Aspergillus flavus*. Additionally, we have

demonstrated that the *A. nidulans gpdA* gene, which encodes glyceraldehyde-3-phosphate dehydrogenase, shows rhythmic expression in constant darkness with a period of approximately 28 hours. Several approaches are currently underway to investigate the circadian clock in *Aspergillus* and these will be presented.

417 **A *STE12* homolog from the asexual, dimorphic fungus *Penicillium marneffei* complements the defect in sexual development of an *Aspergillus nidulans steA* mutant.** Anthony R. Borneman, Michael J. Hynes and Alex Andrianopoulos. Department of Genetics, University of Melbourne. Victoria, Australia. 3010.

Penicillium marneffei is an opportunistic fungal pathogen of humans and the only dimorphic species identified in its genus. At 25 C *P. marneffei* exhibits true filamentous growth while at 37 C, *P. marneffei* undergoes a dimorphic transition to produce uninucleate yeast cells which divide by fission. Members of the *STE12* family of regulators are involved in controlling mating and yeast-hyphal transitions in a number of fungi. We have cloned *stlA*, a homolog of the *S. cerevisiae STE12* gene from *P. marneffei*. The *stlA* gene, along with the *A. nidulans steA* and *Cryptococcus neoformans STE12alpha* genes, form a distinct subclass of *STE12* homologs which have a C_2H_2 zinc finger motif in addition to the homeobox domain that defines *STE12* genes. To examine the function of *stlA* in *P. marneffei*, targeted deletion of the *stlA* gene was used to create a *stlA* mutant strain. Loss of StlA function had no detectable effect on vegetative growth, asexual development or dimorphic switching in *P. marneffei*. Despite the lack of an apparent function, the *P. marneffei stlA* gene complemented the sexual defect of an *A. nidulans steA* mutant and in addition, substitution rate estimates indicate that there is a significant bias against nonsynonymous substitutions between *stlA* and *steA*. These data suggest that *P. marneffei* may have a previously unidentified cryptic sexual cycle in which *stlA* plays a role therefore accounting for the high degree of conservation observed.

418 **Cloning of chitin deacetylase gene from *Phycomyces blakesleeanus.*** Imamura, Kengo[*] , Kondo, Hisae[*], Yoshida, Shuji[*] , Murayama, Tadako[*], Miyazaki Atsushi[**], Otaki, Tamotsu[**] ([*]Coll. of Engn., Kanto-gakuin Univ. , [**]IGE, Tohoku Univ.)

The cell wall of the Zygomycetes fungus *Phycomyces blakesleeanus* is mainly composed of chitin and chitosan. Chitin is converted to chitosan by chitin deacetylase. It has been reported that the biosynthesis of chitosan in these fungi proceeds by the coordinated action of chitin synthase and chitin deacetylase. In the *pilD* mutant which was induced by 4-nitroquinoline-1- oxide and forms swollen and short sporangiophores similar to those of *Pilobolus*, the content of chitin was lower than in the wild type and the content of chitosan was much higher than in the wild type. It suggests that chitin deacetylase activity is higher in the *pilD* mutant than in the wild type. We cloned the genomic DNA fragment (218bp) encoding the homologous amino acid sequence to the consensus sequences in chitin deacetylase of *Mucor rouxii* (*Mr*CDA), nodB protein of *Rhizobium leguminosarum*, and polysaccharide deacetylase of *Bacillus stearothermophilus* by PCR. Southern analysis with this DNA fragment suggested there is only one copy of the chitin deacetylase homologue in this fungus. A positive cDNA clone was isolated from the cDNA libraries made from exponentially growing mycelia of *pilD*. The clone is composed of 1,613bp and encodes a predicted polypeptide (*Pb*CDA) of 459 amino acids. Amino acid sequence of *Pb*CDA is 45% homologous to that of *Mr*.CDA in the central part of the molecule. The sequence analysis of genomic DNAs of the wild type and the *pilD* mutant showed that there is no intron in the coding region of *Pb*CDA gene and there is no difference of the nucleotide sequence in the ORFs between the wild type and *pilD*. These results suggest that *pilD* mutation is located outside the coding region.

419 **The Role of small GTPases in the growth and development of the dimorphic fungus *Penicillium marneffei.*** K.J. Boyce, M.J. Hynes and A. Andrianopoulos. Department of Genetics, University of Melbourne, Royal Parade, Parkville 3010, Australia.

The opportunistic human pathogen *Penicillium marneffei* exhibits a temperature dependent dimorphic switch. At 25 C, *P. marneffei* grows vegetatively as multinucleate filamentous hyphae, which can differentiate to produce asexual spores (conidia). At 37 C, *P. marneffei* grows as uninucleate yeast cells which divide by fission. We are interested in elucidating the pathways regulating dimorphic switching. Small GTPases have been shown to regulate morphological transitions in numerous organisms and we are therefore investigating the role played by small GTPases in *P. marneffei*. Using heterologous probes and degenerate PCR, we have cloned homologues of the Ras and Rho GTPase family from *P. marneffei*. Sequence comparisons with other cloned small GTPase genes has shown that we have isolated a highly conserved *CDC42* (*cflA* = cdc fortytwo like gene A), *Rac* (*cflB* = cdc fortytwo like

gene B) and *RAS2* (*rasA*) homologue. *cflA* was cloned by low stringency hybridisation using the *Aspergillus nidulans modA* gene and encodes a highly conserved *CDC42* homologue. The *cflA* gene expresses two transcripts during vegetative growth, in asexually developing cultures at 25 C and during yeast like growth at 37 C. We have generated dominant negative and activated *cflA* alleles, based on *Saccharomyces cerevisiae CDC42* mutations, and introduced these into *P. marneffei*. Dominant negative transformants have a delay in germ tube emergence, whereas, the dominant activated *cflA* transformants showed an increase in the rate of germ tube emergence. Both dominant negative and activated transformants possessed abnormally swollen and misshapen hyphae. At 37 C, the yeast cells produced by the dominant negative and activated transformants were swollen and misshapen and possessed cell division defects. Therefore, at 25 C, CflA is involved in the control of germ tube emergence and in the maintenance of polarised hyphal growth and at 37 C, CflA controls the morphology of yeast cells. CflA does not affect dimorphic switching in *P. marneffei*, as both transformants were capable of undergoing the morphological transition. Interestingly, the *cflA* dominant negative and activated mutations do not detectably affect conidiation in *P. marneffei*, which is in contrast to *A. nidulans*. Conversely, when either of the mutant *P. marneffei cflA* alleles were transformed into *A. nidulans*, the result was a complete lack of conidiation.

420 **Role of pheromone precursor genes of *Magnaporthe grisea* in mating.** Piotr Bobrowicz, Wei-Chiang Shen, and Daniel J. Ebbole. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA.

Sexual reproduction in heterothallic ascomycetes requires the interaction between two mating partners of the opposite mating type. As was shown in model yeast species the recognition between cells during mating involves a pheromone signaling system. To better understand the role of pheromones and the pheromone response pathway in filamentous ascomycetes we cloned two pheromone precursor genes of an important plant pathogen, *Magnaporthe grisea*. The genes are present in both mating types but they are expressed in a mating type specific manner. *Mat1-1* strains of the fungus express the *MF1-1* gene that is predicted to encode a short polypeptide with a terminal CAAX motif found in lipopeptide pheromones. The *MF2-1* gene, expressed in *Mat1-2* strains, is related in gene organization to the alpha-factor genes of *Saccharomyces cerevisiae* and is probably processed by a Kex2-like protease. Analysis of the null mutants in sexual crosses indicates that pheromones are important, however not essential, for male fertility and that they are dispensable for the female fertility.

421 **TupA, the *Penicillium marneffei* TUP1 homologue, represses asexual development and yeast-like growth to allow vegetative filamentous growth.** Richard B. Todd, Michael J. Hynes and Alex Andrianopoulos. The University of Melbourne, Parkville 3010, AUSTRALIA.

The opportunistic human fungal pathogen *Penicillium marneffei* is dimorphic, showing growth as two different morphological forms. At 25 C the free-living saprophyte grows vegetatively as multinucleate filamentous hyphae and may undergo asexual development (conidiation) by the production of conidia-bearing conidiophores. At 37 C growth occurs as uninucleate yeast cells that are pathogenic. Little is known about the molecular events involved in the establishment and maintenance of the developmental states in *P. marneffei.* We have cloned *tupA,* the homologue of the *Saccharomyces cerevisiae* pleiotropic repressor *TUP1,* from *P. marneffei*. The TupA protein is highly conserved with homologues in other filamentous and yeast- like fungi. Deletion of the *tupA* gene results in pleiotropic effects. The *tupA* deletion mutant shows partial derepression of a subset of carbon catabolite (glucose) repressible metabolic activities, at 37 C formation of irregularly shaped yeast-like cells, and at 25 C inappropriate developmental switching. At 25 C prolific asexual development and production of uninucleate yeast-like cells occurs, indicating that *tupA* plays a role in vegetative filamentous growth by repression of both asexual and yeast developmental programs. These data contrast with both the lack of asexual development and the constitutive filamentation observed in the equivalent mutants in the mono-morphic fungus *Neurospora crassa* and in the dimorphic fungal pathogen *Candida albicans*, respectively. Although TUP1 homologues in fungi are structurally conserved and regulate analogous developmental and metabolic processes they appear to differ in their specific roles within these processes.

422 **Use of GFP variants to study conidial germination in *Colletotrichum gloeosporioides.*** Sima Barhoom, Iris Nesher, and Amir Sharon. Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel

Colletotrichum gloeosporioides f. sp. *aeschynomene* (C.g.a.) is pathogenic on the weed *Aeschynomene virginica*. A biocontrol product named Collego that is used to control *A. virginica* is produced from C.g.a. conidia. The mature conidia are dormant but submerging of conidia in pea extract induces instant germination. Upon induction the conidia divide and then a single germ tube is formed. Staining of cell walls and nuclei revealed that the pea extract promotes fast cytological changes in the conidia. After 1 hour the nucleus divides and then a septum is formed. About 2.5 h after the induction one of the two nuclei is no longer detected by propidium iodide staining while the other nucleus divides, and a germ tube is formed.In the current work we used C.g.a.-transgenic strains that express various forms of GFP to further characterize the germination process. Work with C.g.a.isolates that express YFP in the cytoplasm and GFP in the nucleus revealed new details of the cellular events. Less than 30 minutes after induction the nucleus migrates to one side of the conidium, it then divides and the two new nuclei move each to an opposite pole. A septum is formed and the nuclei return to the center of the cell. Finally, a germ tube and a new nucleus are formed, the new nucleus migrates to the tip of the germ while the nuclei in the original conidial cells start to degrade. The current data suggest that the germination process in C.g.a. may involve a cell cycle controlled germination process in one cell and a programmed cell death in the other.

423 **Isolation and genetic characterization of *Sordaria macrospora* mutants, defective in fruiting body development.** Sandra Masloff, Stefanie Pöggeler, Severine Mayrhofer, Ulrich Kück. Lehrstuhl für Allgemeine und Molekulare Botanik, Fakultät für Biologie, Ruhr-Universität Bochum, D - 44780 Bochum, Germany

The homothallic ascomycete *Sordaria macrospora* shows an apandrous fruiting body formation during sexual development. In order to understand the genetic control of this developmental process, we have started a program to generate mutant strains, which are defective in different steps of fruiting body development. This includes the formation of fruiting body initials, the transition of protoperithecia into perithecia and the maturation of ascospores. Using UV- and EMS-mutagenesis, we have generated a set of 110 mutants, showing different developmental blocks during fruiting body formation. All developmental mutants have in common that they do not show any disposal of mature ascospores through the ostiole (pre-formed opening) of the perithecia. From characterization using scanning and light microscopy, these mutants can be divided into different classes according to their morphological phenotypes: 1. **asc** mutants are characterized by the lack of any ascogon-like structures, which can be considered as the primary initials of fruiting body formation. 2. **pro** mutants form only protoperithecia with a size of about 20-50 µµm. Thus, this type of mutants is unable to form any perithecia or ascospores. 3. **per** mutants are able to generate mature perithecia (size about 500 µµm), however, ascospore formation is completely prevented. These mutants have a defect in meiotic or post-meiotic nuclear division. A detailed analysis providing genetic and morphological data from all mutants will be presented. Allelic tests, for example, showed that beyond this large set of mutants several strains carry allelic mutations, indicating that our attempts are close to a saturated mutagenesis.

424 **Molecular analysis of developmental genes in *Sordaria macrospora*: Identification and characterization of putative components of signal transduction pathways.** Sandra Masloff, Stefanie Pöggeler, Sabine Jacobsen, Henriette Bruggemann, Ulrich Kück. Lehrstuhl für Allgemeine und Molekulare Botanik, Fakultàt für Biologie, Ruhr- Universitàt Bochum, D- 44780 Bochum, Germany

Fruiting body development in the homothallic ascomycete *Sordaria macrospora* is controlled by multiple developmental genes. In order to understand these morphogenic processes on the molecular level, we provide the detailed analyses of genes, which are involved in the transition of protoperithecia into mature fruiting bodies (perithecia). One example is the *pro1* gene, which encodes a putative transcription factor. PRO1 carries a DNA-binding domain, which shows homology to the Zn(II)2Cys6-cluster of other fungal C6 zinc finger proteins. Using a PCR-based cloning strategy, we succeeded in isolating homologues of the *pro1* gene from two related heterothallic species, namely *Neurospora crassa* and *Sordaria brevicollis*. On the DNA level, the genes from the two heterothallic species show a 91 % and a 90 % similarity, respectively, to the *pro1* gene from *Sordaria macrospora*. Amino acid sequence comparisons of the predicted PRO1 polypeptides from the three ascomycetes showed typical DNA-binding domains. We demonstrate by DNA-mediated transformations that the *N. crassa* homologue is able to restore fertility in the sterile *S. macrospora* pro1 mutant. Using *in vitro* mutagenesis, we are able to demonstrate that single nucleotide base pair substitutions in the coding region of the DNA binding domain of the wild type *pro1* gene result in mutant allels, which are unable to restore the sterile phenotype. Fusions of parts of the PRO1-polypeptide to the GAL4 DNA binding domain led to the identification of a domain, which is able to transactivate gene expression

in yeast. The molecular analysis of other developmental mutants with a defective fruiting body formation will be presented.

425 **Meiotic deletion at the *Magnaporthe grisea BUF1* locus is controlled by interaction with the homologous chromosome**, Mark L. Farman. University of Kentucky, Plant Pathology, Lexington, KY, USA

The *Magnaporthe grisea BUF1* gene suffers high frequency mutation in certain genetic crosses, resulting in buff-colored progeny. Analysis of 16 *buf1*$^{-}$ mutants arising from a cross with a mutation frequency of 25% revealed that, in every case, the *BUF1* gene was deleted. The deletions occurred in only one of the parental chromosomes and were due to intrachromosomal recombination. Tetrad analysis revealed that deletions occurred in 44% of meioses and usually affected both chromatids of the mutable chromosome. This suggests they happen before the pre-meiotic round of DNA synthesis. However, they were also almost entirely restricted to crosses in which the homologous chromosome carried the non-mutable allele. This, together with the discovery of extensive structural polymorphism between the stable and unstable *BUF1* loci, suggests that the deletion process is governed by pairing interactions between homologous chromosomes. Given that karyogamy is not supposed to occur until after pre-meiotic DNA replication in Pyrenomycetous fungi like *M.grisea*, this latter observation would place the time of deletion during, or after, DNA synthesis. These conflicting results suggest that karyogamy might actually precede replication in *M.grisea*, or that parts of the genome remain unreplicated until after karyogamy and subsequent chromosome pairing have taken place. Results of experiments to determine the timing of *BUF1* deletion will be presented.

426 **Withdrawn**

427 **The *ubc2* gene of *Ustilago maydis* encodes a novel adaptor protein required for filamentous growth, pheromone response and virulence.** Scott E. Gold, Alfredo D. Martinez-Espinoza and Maria E. Mayorga*. Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274 *Current address: Microbia Inc., 840 Memorial Drive, Cambridge, MA 02139

The Basidiomycete fungus *Ustilago maydis*, causes corn smut disease and alternates between a budding haploid saprophyte and a filamentous dikaryotic pathogen. Previous work demonstrated that haploid adenylate cyclase (*uac1*) mutants display a constitutively filamentous phenotype. Suppressor mutants of a *uac1* disruption strain, named *ubc* for *Ustilago* bypass of cyclase, no longer require cAMP for the budding morphology. The *ubc2* gene was isolated through complementation by virtue of its requirement for filamentous growth. The deduced amino acid sequence encoded by *ubc2* shows localized homology to Sterile Alpha Motif (SAM), Ras Association (RA) and src homology 3 (SH3) protein-protein interaction domains. A K78E missense mutation within the SAM domain, revealed a genetic interaction between *ubc2* and *ubc4*, a pheromone responsive MAP kinase kinase kinase. This indicates involvement of *ubc2* in the pheromone responsive MAP kinase cascade and *ubc2* is required for pheromone responsive morphogenesis. The *ubc2* gene is a critical virulence factor. Thus *ubc2* encodes a novel adaptor protein acting directly upstream of the pheromone responsive MAP kinase cascade in *U. maydis*.

428 **Differential gene expression in dimorphism and pathogenesis in *Ustilago maydis.*** María D. García-Pedrajas, David L. Andrews, Karen E. Snyder and Scott E. Gold. Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274

Ustilago maydis displays dimorphic growth alternating between a budding haploid and a filamentous dikaryon formed by the fusion of two haploid cells. This morphological switch plays a critical role in pathogenicity since only the filamentous dikaryon can infect corn plants. Previously we have identified a role for the cAMP signal transduction pathway in dimorphism and pathogenicity. We are now using suppression subtractive hybridization PCR (SSHP) to identify novel genes involved in dimorphism. We have identified several genes upregulated during filamentous or budding growth and have confirmed differential expression by northern blot analysis. We are now in the process of fully characterizing some of these genes. We plan the production of disruption mutants to determine the biological roles of select genes in morphogenesis and/or pathogenesis. We are also using the same technique, SSHP, to identify genes from both the plant and the fungus involved in gall formation and teliosporogenesis. In order to analyze a large number of clones after the construction of the subtractive cDNA libraries, we are using

robotic devices to automatically pick and array colonies on nylon membranes. In this way we can efficiently analyze several thousand colonies simultaneously.

429 **Suppressors of the meiotic defects of *spo11-1* in *Coprinus cinereus.*** Martina Celerin, Jana Stone, Rachel Shiffrin, Kelly K. Meeks, Sandra T. Merino, Miriam E. Zolan. Department of Biology, Indiana University, Bloomington, Indiana, 47401

In the mushroom caps of wild-type *Coprinus cinereus*, the basidial nuclei undergo meiosis, and each of the four products migrates into a basidiospore. These basidiospores give the cap its black appearance. In contrast, the *C. cinereus spo11-1* mutant is defective in meiosis and produces a virtually sporeless cap, which is white. Spo11, a type II topoisomerase, likely is required universally for the initiation of meiotic recombination, and *C. cinereus spo11-1* fruits white. We screened for genetic suppressors of the spore formation defect by mutagenizing oidia from *spo11-1* and looking for surviving strains that were able to produce basidiospores. Of the 1385 isolates that produced fruiting bodies, eight produced grey caps. All eight produced more spores than the *spo11-1* mutant, and the percent viability of these spores was higher than that of the starting strain. These eight mutants represent four complementation groups, termed *sse1-sse4*, for **s**uppressors of ***s**po **e**leven*. Previous studies have shown that suppression of *spo11-1* by artificially created DNA breaks (caused by ionizing irradiation) or by the absence of a sister chromatid induce formation of synaptonemal complex (SC). Surprisingly, none of the suppressors isolated in this study induces substantial amounts of SC formation in a *spo11-1* background. Thus, they presumably suppress the sporulation defects in *C. cinereus spo11-1* by completely different mechanisms.

430 **Factors affecting meiotic stability of the *BUF1* gene in *Magnaporthe grisea.*** Yun-Sik Kim and Mark L. Farman. University of Kentucky, Plant Pathology, Lexington, KY, USA

Meiotic stability of the *BUF1* gene in *Magnaporthe grisea* strain Guy11 is controlled by interaction with the homologous chromosome and appears to be correlated with the homologous *BUF1* loci having different physical organizations (see abstract by M. Farman). In this abstract, we describe the testing of two hypotheses related to *BUF1* instability: Hypothesis 1: *BUF1* instability is caused by mis-pairing between the homologous *BUF1* loci. Physical mapping was used to identify strains with different physical organizations at their *BUF1* loci. Genetic crosses were then used to incorporate these loci into both the *MAT1-1* and *MAT1-2* mating type backgrounds. Compatible strains were then mated so that the loci were paired in all possible combinations. Regardless of origin, the *BUF1* gene was stable in most crosses involving *BUF1* loci with identical organizations. In the one exceptional cross it was lost at a low frequency. Loci that were originally from strains 2539 and Arcadia were perfectly stable in all crosses. However, the loci from ML33 and Guy11, which both suffered deletion in crosses with strain 2539, were stable in crosses with Arcadia. Arcadia has a *BUF1* locus structure that is quite different from that of ML33 or Guy11, indicating that mis-pairing *per se* is insufficient to induce instability at the *BUF1* locus. Instead, we propose that specific structural differences promotes the loss of *BUF1*. Hypothesis 2: The frequency of deletion is determined by the physical organization of the *BUF1* locus. This was tested by crossing strains with different *BUF1* locus structures to the same tester strain and measuring deletion frequency. Surprisingly, the frequency varied significantly between different crosses of the same strains. Furthermore, if the *BUF1* gene survived deletion through crosses, it became progressively more stable in each subsequent generation. This suggests that *BUF1* deletion frequency is determined primarily by genetic background and/or environmental factors.

431 **Two G-alpha proteins, GNA-1 and GNA-3, regulate adenylyl cyclase in *Neurospora crassa*.** Ann M. Kays, F. Douglas Ivey, and Katherine A. Borkovich. University of Texas Health Science Center - San Antonio and University of Texas - Houston Medical School.

Cellular responses to external stimuli, such as odorants and pheromones, are mediated by heterotrimeric G protein coupled receptors in eukaryotic systems. Our laboratory has identified and characterized three G alpha subunit genes, *gna-1*, *gna-2*, and *gna-3*, from the saprophytic fungus *Neurospora crassa*. *N. crassa* G alpha proteins display remarkable conservation of sequence and function with G alpha proteins from the pathogenic fungi *Magnaporthe grisea* and *Ustilago maydis*. All three G alpha subunits have been shown to regulate different aspects of sexual development. GNA-1 is a positive regulator of apical extension and GNA-3 is a negative regulator of asexual sporulation. Deletion of *gna-1* in an adenylyl cyclase null mutant confers increased thermotolerance and elevated expresssion of stress proteins. Biochemical analysis indicates that GNA-1 and GNA-3 differentially regulate

adenylyl cyclawe activity and levels, respectively. A delta *gna-1* delta *gna-2* delta *gna-3* and a delta *gna- 1* delta *gna-3* mutant have been constructed by a sexual cross to explore the role of each G alpha protein in signaling. Studies exploring the roles of these mutants in *Neurospora crassa* development will be presented.

432 A**nalysis of two genes that are highly expressed in starved and sexual tissues of *Neurospora crassa***. Hyojeong Kim and Mary Anne Nelson. The University of New Mexico, NM, USA

Two novel and highly expressed genes were identified by the Neurospora Genome Project at UNM (Nelson et al., Fungal Genetics and Biology 21, 348-363, 1997). These genes are tentatively named poi-1 and poi-2 (for plenty of it), since they are the most highly expressed genes in the starved mycelial and/or perithecial tissues of N. crassa. The most abundantly expressed of the two genes, poi-1, is expressed as an approximately 1.2 kb transcript and contains an ORF of 24 aa near the 5' end. The mostly non-coding poi-1 mRNA is predicted to form an unusual secondary structure. Analysis of the poi-1 mutants generated by repeat-induced point mutation (RIP) shows that not only the ORF but also the rest of the transcript is required for normal vegetative and sexual development. The second most abundantly expressed gene, poi-2, contains an ORF with good codon bias. It has been shown by in vitro transcription and translation to encode a 27 kDa protein, and the putative protein is predicted to contain a transmembrane helix and a signal peptide. The putative poi-2 protein also contains a novel 16 tandem repeat of 13-14 amino acid residues, the partial loss of which results in defective vegetative development.

433 **Etiology of senescence associated with retroplasmid-containing strains of *Neurospora***. A. Nicole Fox, Jonamani Saud and John C. Kennell. Department of Biological Sciences, SMU, Dallas, TX

Senescence of filamentous fungal cultures is invariably associated with mitochondrial dysfunction. Unlike *Podospora*, in which senescence appears to be part of the life cycle, growth decline in *Neurospora* is uncommon and generally limited to strains that harbor certain mitochondrial plasmids. We have been studying the etiology of senescence in strains of *Neurospora crassa* containing the Mauriceville mitochondrial retroplasmid. Previous studies have shown senescent cultures contain variant forms of the retroplasmid that contain cDNA copies of mt tRNAs. Here, we evaluate the rate at which variant plasmids arise in subcultures of the Mauriceville strain and their association with the senescent phenotype. Although variant plasmid formation always preceded senescence, subcultures were found to tolerate the variant plasmids for variable lengths of time and no correlation could be made between the specific sequence inserted in the plasmids and the rate or frequency of senescence. We also report the isolation of a strain in which senescence occurs in the absence of variant plasmid formation or detectable alterations in mtDNA. Spectral analysis of mitochondria isolated from pre-senescent cultures show greatly reduced levels of cytochrome *c* and genetic studies indicate the senescent phenotype is associated with a single nuclear mutation. Interestingly, the mutation appears to function in a dominant negative fashion. Together with other studies, our findings indicate that there are at least three separate pathways which lead to senescence in *Neurospora*.

434 ***A. nidulans Ste*A a Homeodomain/Zn^{+2} finger protein binds DNA by its homeodomain.** Marcelo A. Vallim[1,2] and Bruce L. Miller[2]. Universidade de Sao Paulo, Faculdade de Ciencias Farmaceuticas de Ribeirao Preto, Ribeirao Preto-SP, 14040-903. Brazil[1]. University of Idaho, Microbiology, Molecular Biology and Biochemistry Depart., Moscow, ID 83843,USA[2]

The homeodomain (HD) protein Ste12p plays a key role in determining cell identity, mating response and dimorphic transitions in *S. cerevisiae* by coupling MAPKinase signal transduction pathways to gene-specific transcriptional activation. Ste12p homologues have been identified in other dimorphic yeast, and its has been reported to be required for invasive, filamentous growth and pathogenesis. *ste*A, a *ste*12 homologue, was isolated from the homothallic, filamentous fungus *A. nidulans* (Vallim *et al*., 2000, Mol. Microbiol. 36:290-301,) SteAp has an N-terminus HD similar to Ste12p, and in add tion, it has a C-terminus C2/H2-Zn^{+2} finger domain. Inactivation of *ste*A resulted in sterility and inability to differentiate ascogenous tissue and cleistothecia, but did not interfere with either filamentous growth or polymorphic transitions associated with conidiation. Here we report the results of an oligonudeotide library screening, which shows that SteAp HD actually binds specifically to a DNA sequence with 7 bases, *in vitro*. A strong transcription activation domain was characterized at the N-terminus portion of SteAp by a yeast two-hybrid assay. The transcription of *med*A and *stu*A which are absent in the fungus hyphal stage, are de-repressed in the disrupted *ste*A backgrond. However, gel shift assays using *med*A and *stu*A promoter sequences showed that they carry no target for the homeodomain. *In vivo* assays showed that over expression of SteAp

inhibited the expression of a reporter gene placed downstream the promoter sequence of stuA during develpoment suggesting that SteAp might be involved in activating the transcription of a factor engaged in repressing the transcription of this gene.

435 **Components and function of the Fus3/Kss1-related MAP kinase pathway in *Neurospora crassa*.** Piotr Bobrowicz and Daniel J. Ebbole. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA.

In yeast *Saccharomyces cerevisiae* two closely related MAP kinases Fus3p and Kss1p regulate pheromone response and filamentous growth. Recently, close homologues of those kinases were isolated in several pathogenic filamentous ascomycetes and shown to be important pathogenicity factors. Compared to the situation in yeast our knowledge about signaling pathway(s) regulated by those proteins and their target genes is still very limited. We decided to use *Neurospora crassa*, a well-characterized heterothallic ascomycete, to isolate more components of the Fus3/Kss1-related MAP kinase pathway and try to identify genes regulated by it. The MAP kinase similar to yeast Fus3p and Kss1p is encoded in *N. crassa* by the *mak-2* gene. The *mak-2* gene replacement mutants show significant reduction in growth rate and have poorly developed aerial hyphae. Mutants are fertile as a male but fail to produce protoperithecia and therefore are completely sterile as a female. Ascospores with deleted *mak-2* gene do not germinate. Initial results from Western blot experiments indicate that *mak-2* gene might be the only *FUS3/KSS1* homologue in *N. crassa*. Using subtractive cloning we identified 5 novel genes that are not express or show reduced expression in *mak-2* mutant as well as 2 genes that are overexpressed in mutant. The second gene from the pathway that we currently analyze is the *STE12* homologue. The sequence of the predicted protein is very similar to the SteAp from *Aspergillus nidulans* with homeodomein in N-terminal part and C-terminal C2/H2 zinc finger motif. The characterization of this gene will determine if there is an epistatic relationship with *mak-2*.

436 **Roles of NC-*ras2* in apical growth of *Neurospora*.** Tadako Murayama, Junko Yoshie and Junichi Kawakami (Kanto-gakuin Univ. Yokohama, Japan)

A morphological mutant *smco7* is a null mutant of one of the *ras* homologues in *Neurospora*, NC-*ras2*. The extension growth of the *smco7* mutant was considerably lower than that of the wild type. The hyphae of the mutant were thinner and more crowded than those of the wild type. The apical cells of *smco7* were shorter, thinner, and more fragile than those of the wild type. The *smco7* mutation seemed to cause the defects in cell wall synthesis. The cell wall precursors in the apical vesicles have been reported to be transported to the apices, and secreted there through the common mechanisms to those in the transport and exocytosis of vesicles containing extracellular enzymes. The extracellular invertase and trehalase activities were much lower in *smco7* than in the wild type. An actin inhibitor, Cytochalasin A (CA), considerably inhibited the hyphal growth, made hyphae thinner and more crowded, and lowered the level of extracellular invertase after the mycelia of the wild type were shifted to the medium containing CA. The *smco7* mutant was much more sensitive to CA than the wild type. These results suggest that the actin plays important roles in the apical growth of the hyphae and the secretion of extracellular enzymes and the NC-*ras2* protein plays some roles in the regulation of function of actin in *Neurospora*. The relationship between NC-*ras2* and *mcb* which has been suggested to regulate growth polarity through organizing actin patches at the cell cortex will be discussed.

437 **A homolog of the transcriptional repressor Ssn6p antagonizes cAMP signalling in *Ustilago maydis*.** Michael Feldbrüggee[1], Gabriel Loubradou[2], Andreas Brachmann[2], and Regine Kahmann[1]. [1]Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., D-35043 Marburg, [2]Institute of Genetics and Microbiology, Ludwig Maximilians University, Maria-Ward-Str. 1a, D-80638 München

In *Ustilago maydis* cAMP signalling is crucial for successful infection of maize plants. Strains are nonpathogenic if mutated in all of the so far identified components of the cAMP signalling pathway. Deletion of signalling components resulting in low cAMP levels trigger filamentous growth and high cAMP conditions cause the formation of glossy colonies such as conferred by the allele $gpa3_{Q206L}$ encoding a constitutively active form of the G alpha subunit. By screening a multicopy plasmid library in $gpa3_{Q206L}$ mutant cells we identified *sql1* as suppressor of the glossy colony phenotype. Interestingly, only alleles encoding a C terminal truncated version of Sql1 were able to complement the mutant phenotype. Sql1 is a functional homologue of the yeast transcriptional repressor Ssn6p and contains 10 TPRs of which the first six are important for suppressor function. Wild type strains expressing various C

terminal-truncated versions that are able to complement glossy colonies of $gpa3_{Q206L}$ show filamentous growth in liquid culture. Filament formation is reversed in the presence of cAMP. According to our model Sql1 is part of an evolutionary conserved Sql1/Tup1 transcriptional repressor complex that antagonizes cAMP signalling by repressing cAMP-regulated genes.

438 **A pcl-like cyclin of *Aspergillus nidulans* is transcriptionally activated by developmental regulators and is involved in sporulation.** Niklas Schier, Ralf Liese and Reinhard Fischer. Philipps-Universitat Marburg and Max-Planck-Institut fur terrestrische Mikrobiologie, Marburg, Germany.

The filamentous fungus *Aspergillus nidulans* reproduces asexually through the formation of spores on a multicellular aerial structure, called conidiophore. A key regulator of asexual development is the TFIIIA-type zinc finger containing transcriptional activator Bristle (BRLA). Besides BRLA, the transcription factor ABAA, which is located downstream of BRLA in the developmental regulation cascade is necessary to direct later gene expression during sporulation. We isolated a new developmental mutant and identified a leaky *brlA* mutation and the mutated *Saccharomyces cerevisiae* cyclin homologue *pclA*, both contributing to the developmental phenotype of the mutant. *pclA* was found to be 10-fold transcriptionally upregulated during conidiation and a *pclA* deletion strain was 3-5-fold reduced in conidia production. Expression of *pclA* was strongly induced by ectopic expression of *brlA* or *abaA* under conidiation suppressing conditions indicating a direct role for *brlA* and *abaA* in *pclA* regulation. PCLA is homologous to yeast Pcl cyclins, which interact with the Pho85 cyclin-dependent kinase. However, PCLA function during sporulation was independent of the *A. nidulans* Pho85 homologue PHOA. Besides the developmental regulation, *pclA* expression was cell cycle-dependent with peak transcript levels in S-phase. Our findings suggest a role for PCLA in mediating cell cycle events during late stages of sporulation. To identify interacting proteins of PCLA, especially the cyclin-dependent kinase, a Two-Hybrid screening and biochemical approaches are underway.

439 **Mating-type genes control fertilization, internuclear recognition, developmental arrest and recovery in *Podospora anserina*.** Sylvie Arnaise, Evelyne Coppin, Robert Debuchy, Denise Zickler and Marguerite Picard. Institut de Génétique et Microbiologie, UMR 8621, bâtiment 400, Université Paris-Sud, F-91405 Orsay cedex, France

A common feature of the sexual cycle of fungi is the formation of a large number of different progeny from one mating event. This feature is achieved in Euascomycetes after fertilization, by a series of mitotic divisions of parental nuclei within a syncytium. The development proceeds then to a transition from the syncytial stage to a cellular stage, which requires that a male and a female nucleus recognize each other (internuclear recognition, IR) and migrate from the plurinucleate cell into a specialized hypha where they divide synchronously. Caryogamy, meiosis and ascospore formation take place within these specialized hyphae. The heterothallic euascomycete *Podospora anserina* has two idiomorphs, *mat+* and *mat-*, which control the two recognition steps of the sexual cycle: fertilization and IR. Fertilization is regulated by *FMR1* (*mat-* specific gene) and *FPR1* (*mat+* specific gene). Mutations in *FMR1* indicate that it operates as an activator of *mat-* fertilization functions and repressor of *mat+* fertilization functions. *FPR1* has opposite functions in *mat+* strains. IR is under the control of three regulatory genes: *FMR1* and *SMR2* (*mat-* specific genes), and *FPR1* (*mat+* specific gene). Mutations in these genes suggest that *FMR1* and *SMR2* operate as activators of the *mat-* IR system and repressors of the *mat+* IR system in *mat-* nuclei, whereas *FPR1* have reversed effects in *mat+* nuclei. Theses results are in agreement with a model of IR based on a nucleus-limited expression of these genes. Vegetative overexpression of the IR regulatory genes suggests that they also induce a developmental arrest which is overcome by the expression of the third *mat-* gene, *SMR1*.

440 **Genetic interactions between a mating-type gene and a gene involved in nuclear distribution in *Podospora anserina*.** Khaled Bouhouche, Sylvie Arnaise, Denise Zickler and Robert Debuchy. Institut de Génétique et Microbiologie, UMR 8621, bâtiment 400, Université Paris-Sud, F-91405 Orsay cedex, France.

In *Podospora anserina*, sexual reproduction is under the control of one locus with two alternate alleles *mat-* and *mat+*. Fertilization leads to the formation of plurinucleate cells with intermingled parental *mat+* and *mat-* nuclei. Further development requires an internuclear recognition (IR) between *mat+* and *mat-* nuclei so that *mat+/mat-* pairs migrate into specialized hyphae, that develop in croziers, in which caryogamy, meiosis and ascospore formation take place. *FPR1* (*mat+* specific gene), *FMR1* and *SMR2* (*mat-* specific genes) control IR step, which is followed by a developmental arrest. *SMR1* (*mat-* specific gene) is required for recovery from the block in

development. The *ami1* gene is the functional homologue of the *Aspergillus nidulans apsA* gene (Gra a *et al*, 2000, Genetics, 155, 633-646). *ami1* is involved in nuclear distribution events throughout the life cycle. A loss of function of *ami1* causes abnormal distribution of nuclei in microconidia and mycelial filaments. A loss of function of *ami1* in both parents does not preclude the production of a progeny but frequently causes abnormal distribution of nuclei in crozier cells and asci. Genetic interactions between *ami1* and the *mat* genes have been analyzed in double mutant strains. Preliminary results indicate that a *fmr1-r* mutation, leading to self-recognition of *mat-* nuclei at the time of IR, is epistatic on the *ami1-2* mutation (partial deletion of *ami1*). Surprizingly, association of the *ami1-2* and *smr1-r* mutations leads to complete arrest of the development of the perithecia which look like unfertilized prothoperithecia, whereas the *smr1-r* mutant is able to start fruiting body development and displays an arrest of development before the formation of ascogenous hyphae. This suggests that *SMR1* and *ami1* participate to the same process during early development of the perithecium.

441 Withdrawn

442 **Forcible discharge of ascospores.** F. Trail, H. Xu, I.Gaffoor, and C. Andries. Department of Botany and Plant Pathology, Michigan State University, E. Lansing MI

In many Pyrenomycetes and Loculoascomycetes, ascospores are forcibly discharged from perithecia. The mechanism of forcible discharge of ascospores has not been well studied in any ascomycetous fungus. It is clear that osmotic pressure builds up within the ascus and serves as the force behind discharge. Physiological and genetic methods have been used to elucidate this mechanism. Physiological studies in *Gibberella zeae* have identified several factors that are likely involved in this increase in pressure. The genetic basis of these factors is being studied. Generation of 5000 insertional mutants yielded one that formed morphologically normal perithecia that had lost their ability to discharge ascospores. Analysis of the discharge minus mutant is in progress.

443 **Characterization of the *racA* gene in *Aspergillus niger*.** A.F.J. Ram[1,2], M. Arentshorst[1], R.A. Damveld[1], P.J. Punt[2] and C.A.M.J.J. van den Hondel[1,2]. [1]Leiden University, Inst. Mol. Plant Sciences, Leiden, The Netherlands,[2]Department of Appl. Microbiol. Gene Techn., TNO- Nutrition, Zeist, The Netherlands.

The members of the Rho-subfamily of Ras related GTP binding proteins (Rho, Rac and Cdc42) are signalling molecules originally identified as regulators of actin cytoskeleton reorganization. More recent evidence has implicated a wider function of the rho-subfamily that also include activation of kinase cascades and regulation of enzymes activities. Using PCR-based cloning approaches we searched for Cdc42-like GTPases in *A. niger*. Both a Cdc42 (Cdc42A) homologue and a Rac homologue (RacA) were isolated. The presence of a Rac homologue seems to be unique for filamentous fungi, since homologues of this gene are not present in yeasts like *S. cerevisiae* and *S. pombe*. Deletion of the *racA* showed that the gene is not essential. The mutant showed alterations in hyphal morphology and a reduced conidiation. Overexpression of the *racA* gene from the inducible glucoamylase promoter also resulted in reduced conidiation, indicating a role for RacA in conidiophore initiation or formation. A GFP-RacA fusion protein has been constructed which is currently used to localize RacA in the fungal hyphae.

444 **The role of beta-1,3-glucanase in *Phytophthora infestans.*** Adele McLeod, Christine D. Smart, and William E. Fry. Department of Plant Pathology, Cornell University, Ithaca, NY 14853

Phytophthora infestans is an oomycete pathogen that causes late blight of potato and tomato. Oomycetes have a cell wall that is comprised of 80-90% beta-1,3-glucan, therefore beta-1,3-glucanases could play an important role in the biology of these organisms. Suggested functions of beta-1,3-glucanase are the hydrolyses of cell-associated reserve material, hydrolysis of extra-cellular nutrients in the environment, cell wall growth and extension, breakdown of plant cell walls and breakdown of plant defense structures. The aims of our study are to characterize beta-1,3-glucanases from *P. infestans* and to attempt to investigate their biological function in the pathogen by gene silencing. Two putative beta-1,3-glucanase fragments, one endo-beta-1,3-glucanase and one exo-beta-1,3-glucanase, were amplified from *P. infestans* genomic DNA. The putative endo-beta-1,3-glucanase fragment had 35% amino acid similarity to a cell wall endo-beta-1,3-glucanase of *Saccharomyces cerevisiae*, and the putative exo-beta-1,3-glucanase fragment had 45% amino acid similarity to an exo-1,3-beta-glucanase from *Agaricus bisporus*. Southern analysis has shown that there is only one copy of the endo-beta-1,3-glucanase and possibly two copies of the exo-

1,3-beta-glucanase in *P. infestans*. Homologous copies of both genes were found in *P. palmivora* and *P. sojae*. The PCR products of the putative genes were used to isolate clones from a genomic library of *P. infestans*, and we are currently identifying full-length genomic sequences. In vitro analysis of expression of the putative exo- and endo-glucanase genes has shown that they are more strongly expressed in mycelia than in sporangia, zoospores or germinating cysts. Both genes are expressed in planta, and can be detected at 72 h after inoculation.

445 **A Gbeta Protein from *Neurospora crassa.*** Sheven I. Poole, Qi Yang and Katherine A. Borkovich. Univ. Texas-Houston Medical School, Micro. and Molec. Genet., Houston, TX, USA

Heterotrimeric G protein-mediated signal transduction pathways allow cells to respond to environmental stimuli. Accumulating evidence suggests that G protein beta-gamma dimers as well as Galpha subunits play pivotal roles in these pathways. We have identified a Gbeta subunit from *Neurospora crassa*, *gnb-1*. The predicted amino acid sequence of GNB-1 is most identical to *Cryphonectria parasitica* CPGB-1 (91%) and Gbeta proteins from other fungi. RFLP mapping showed that the *gnb-1* gene is located on the right arm of chromosome III near the *con-7* and *trp-1* genes. Northern analysis reveals two *gnb-1* specific transcripts of 2.7 and 1.4 kb. *gnb-1* mutants possess many defects, including aberrant growth in submerged culture and female sterility. Loss of *gnb-1* also impacts levels of Galpha proteins in *N. crassa*.

446 **Mating-regulated gene expression in *Phytophthora infestans.*** Anna-Liisa Fabritius and Howard, S. Judelson. Department of Plant Pathology, University of California, Riverside, CA 92521, USA.

Interactions between the two mating types (A1 and A2) of the heterothallic oomycete, *Phytophthora infestans,* result in the formation of gametangia and the sexual spores, which are called oospores. Genes differentially expressed during sexual development were identified by suppression subtractive hybridization-polymerase chain reaction. Nine genes were identified that were induced during mating. Of these, sequence analysis indicated that one was very similar to a previously identified extracellular glycoprotein elicitor gene of *P. megasperma.* Another gene was highly similar to the pumilio gene of *Drosophila melanogaster,* which encodes an RNA binding protein that regulates early embryogenesis. Time course analysis indicated that three of the mating-regulated genes of *P. infestans* were induced very early in sexual development, before defined male- or female-specific structures appeared. The other six genes were induced during later stages, which corresponded to oospore development and maturation. The mating-induced genes were also expressed in self-fertile strains of *P. infestans.* For one gene, multiple transcripts were detected which showed differential regulation during time-course and between normal matings and self-fertiles. Experiments aimed at silencing the genes in transformants are underway to address their function. Preliminary experiments revealed serious defects in mating behavior among the transformants.

447 **Are *Gibberella zeae* sexual spores the critical inoculum for wheat head blight?** Daren W. Brown[1], Sung-Hwan Yun[2], Theresa Lee[3], B. Gillian Turgeon[4], and Anne E. Desjardins[1]. [1]Mycotoxin Research Unit, NCAUR, USDA/ARS, 1815 N. University St., Peoria, IL 61604. [2]Division of Life Sciences, Soonchunhyang University, Asan, Choongnam, 336-745, Korea. [3]School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea [4]Department of Plant Pathology, Cornell University, 334 Plant Science Building, Ithaca, NY 14853

Gibberella zeae (anamorph *Fusarium graminearum*) causes head blight (scab) in wheat and barley, and ear rot in corn. Since 1991, epidemics of *Gibberella* head blight have had disastrous effects on wheat and barley production in the Midwestern states. In addition to decreasing yields, the fungus also contaminates grain with trichothecene mycotoxins that are harmful to human and animal health. To understand and control head blight, the factors and conditions that lead to epidemics must be identified. Observations in the field suggest that *G. zeae* sexual spores (ascospores) produced on plant debris in the soil are a primary source of inoculum for head blight epidemics. Sexual reproduction in *G. zeae* is controlled by a mating-type (MAT) locus that contains four genes located within 6 kb of genomic sequence. To test the role of ascospores in causing blight, we have constructed an ascospore non-producing strain by deleting the MAT locus using a transformation mediated gene replacement strategy. MAT-null strains appear indistinguishable from wild-type strains in morphology and production of asexual spores (macroconidia). In addition, MAT-null strains are similar to wild-type strains in virulence following injection of individual wheat heads with macroconidia. Experiments planned for Spring 2001 will test a MAT-null strain and the wild- type strain from which it was derived for their ability to cause head blight on wheat following application of infested maize stalk pieces to the soil surface. If ascospores are the major inoculum source, then we predict that exposure of wheat heads

to a MAT-null strain that cannot produce ascospores should cause less blight than exposure to an ascospore producing strain.

448 **Isolation and expression of a gene encoding the plasma membrane H^+-ATPase from the oomycete, *Phytophthora infestans*, the causal agent of potato late blight.** Christine D. Smart[1], Nobutaka Kato[2], William E. Fry[1], and Lawrence B. Smart[2]. [1]Department of Plant Pathology, Cornell University, Ithaca, NY 14853 [2]Faculty of Environmental and Forest Biology, SUNY College of Environmental Science and Forestry, Syracuse, NY 13210

Phytophthora infestans produces asexual sporangia that can germinate either by forming a germ tube directly or by producing motile zoospores. Progression through the various stages of the pathogen life cycle, including differentiation and germination of zoospores, is apparently regulated by ion flux (K^+ and/or Ca^{2+}) across the plasma membrane. These fluxes are, in turn, dependent upon the activity of the plasma membrane (PM) H^+-ATPase. In order to gain a better understanding of the regulation of ion transport through *P. infestans* development, we have initiated molecular studies of the PM H^+-ATPase. We utilized degenerate oligonucleotide primers derived from conserved regions of plant H^+-ATPases for PCR, then used that PCR product as a probe in screening a genomic library of *P. infestans* isolate US940480 (a member of the US-8 clonal lineage). The PM H^+-ATPase in *P. infestans* appears to be encoded by a single gene, which is most similar to genes from algae and to a lesser degree from plants. This gene apparently lacks introns and does not contain consensus eukaryotic core promoter elements. We are characterizing the expression of the PM H^+-ATPase gene under various pH levels and in the presence of different ion concentrations with the hope of gaining further insight into the developmental biology of this oomycete.

449 **Identification of the *STE20a* gene of *Cryptococcus neoformans* reveals that serotype A MATa strains still exist.** Klaus Lengeler and Joseph Heitman. Duke University Genetics

The mating-type loci of the opportunistic human fungal pathogen *Cryptococcus neoformans* are quite unusual in size, spanning ~50 kb, and encode several allelic but divergent genes, including *STE20a* and *STE20alpha*. *C. neoformans* is classified into three varieties: *neoformans* (serotype D), *grubii* (serotype A) and *gattii* (serotype B and C). Interestingly, environmental and clinical isolates are predominantly of MATalpha mating-type, and MATa strains of variety *grubii* (serotype A) were thought to be extinct. We recently identified the *STE20a* gene of serotype D and showed that the gene cosegregates with the MATa mating-type in genetic crosses, maps within the mating-type locus on a 1.8 MB chromosome, and is allelic with the MATalpha locus. During the characterization of unusual clinical isolates we identified a novel *STE20* allele from the clinical isolate 125.91. The novel *STE20* allele is MATa mating-type specific but not identical to the *STE20a* gene from serotype D. Capsular antigen analysis, Southern blot analysis, and DNA sequence comparisons revealed that strain 125.91 is serotype A. We characterized clinical and environmental isolates of an unusual serotype AD and find that these strains are aneuploid or near diploid. In addition, we find these strains are heterozygous for the mating-type locus, suggesting that serotype AD strains arise from intervariety crosses between serotype A and serotype D. Moreover, several serotype AD strains inherited the MATa mating-type locus from a serotype A parental strain, suggesting that fertile serotype A MATa strains exist in nature. The serotype A MATa strain 125.91 is sterile under all conditions tested thus far. In addition, the dissection and characterization of basidiospores derived from a self-fertile serotype AD strain that inherited the serotype A MATa locus did not result in the isolation of a fertile serotype A MATa strain. Our long term goal is to construct a congenic pair of fertile serotype A MATa and MATalpha strains for use in genetic analysis of *C. neoformans* variety *grubii* .

450 **A novel gene from *Neurospora crassa* involved in sexual development is a member of conserved group of protein methyltransferases.** Piotr Bobrowicz and Daniel J. Ebbole. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA.

Recently a number of genes encoding members of a conserved family of protein methyltransferases was characterized and shown to play important role in cell cycle control and cellular signaling. For example the product of the human JBP1 gene interacts with Jak kinases involved in cytokine signal transduction. The Hsl7p of Saccharomyces cerevisiae was found to be a component of the Swe1p/Cdc28p morphogenesis checkpoint. Additionally, Hsl7p is a negative regulator of filamentous growth MAP kinase pathway and was shown to compete with Cdc42p for binding to the Ste20p. Using the *HSL7* sequence to search the sequence generated by the German *Neurospora* sequencing project we identified a close homologue of this gene. The predicted protein contains a

conserved GXGRGP motif specific for protein methyltransferases that is also present in both JBP1 and Hsl7p. The *pp-2* (protoperithecia 2) RIP mutant strains have a slight growth defect and do not produce protoperithecia. Based on the yeast model we have initiated experiments to test the hypothesis that *pp-2* is involved in the control of MAP kinase signaling pathway identified by us in *N. crassa*.

451 **Cloning of pheromone genes in *Podospora anserina*.** Evelyne Coppin and Robert Debuchy. Institut de Genetique et Microbiologie, UMR 8621, batiment 400, Universite Paris-Sud, F-91405 Orsay cedex, France

The development of the fruiting body and the production of a progeny in heterothallic Euascomycetes rely on two recognition steps: an intercellular recognition between compatible reproductive organs, which leads to fertilization and then an internuclear recognition (IR) between compatible nuclei. This latter event occurs in plurinucleate cells which contains female nuclei and their responsive nuclei which are derived from the fertilizing male nucleus by mitotic divisions. The compatible nucleus couples migrate from the plurinucleate cell into a specialized hypha (the ascogenous hypha) where take place karyogamy, meiosis and ascospore formation. The heterothallic euascomycete *Podospora anserina* has two idiomorphs which control fertilization and IR. As idiomorphs encode transcriptional factors, the mechanism of IR remains an enigma. It has been proposed that pheromone - receptor systems may be involved in IR for the production of a cue which would trigger the migration of compatible nuclei into the budding ascogenous hypha. In order to test the role of pheromones in IR, we have decided to clone the pheromone genes in *P. anserina*. Degenerate primers have been designed from the alignment of *Neurospora crassa mfa* and *Magnaporthe grisea MF1-1* pheromone genes. Low stringency PCR with these primers has allowed us to isolate a pheromone gene in *P. anserina*. Its functions are under investigation. Cloning of a pheromone gene similar to *ccg4* of *N. crassa* is in progress by heterologous hybridization. Aknowledgement : we are greatly indebted to Dan Ebbole for communicating the sequence of *mfa* prior to publication and for the gift of plasmids containing *mfa* and *ccg4*.

452 **Inhibition of a serine protease disrupts development in *Cryphonectria parasitica*.** Patricia M McCabe and Neal K Van Alfen, University of California, Department of Plant Pathology, Davis, CA. 95616, USA.

Infection of the chestnut blight fungus, *Cryphonectria parasitica* by a dsRNA virus (CHV1) results in loss of development, which is manifested as a reduction in pigmentation, conidial formation, sexual reproduction and virulence. However, there is no change in growth rate and the fungus remains in a healthy juvenile state. Genes that have been identified based on differential expression between CHV1 infected and wild type strains are secreted proteins that play a role in development. The best characterized are a laccase, a mating type specific pheromone and the hydrophobin cryparin. All of the proteins have identical signals for post-translational processing and are thought to be secreted via a vesicle mediated secretory pathway. CHV1 infection causes the accumulation of fungal vesicles inside the cell. Virus dsRNA and RNA-dependent RNA polymerase activity co-purify with these vesicles. One CHV1 down regulated host protein, cryparin, is also associated with this vesicle fraction. Using pulse-chase analysis to study cryparin secretion we found that CHV1 infection reduced the rate of cryparin secretion through cells. This decrease in secretion rate can be mimicked by the addition of a specific serine protease inhibitor. The inhibitor not only slows the rate of cryparin secretion to levels similar to that of CHV1 infection, but phenotypically mimics the virus infection symptoms i.e. it prevents *C. parasticia* pigmentation and conidia formation without affecting growth, and down-regulates cryparin gene transcription. These remarkable phenotypic similarities suggest that CHV1 is causing symptoms by interfering with a serine protease processed protein secretory pathway.

453 **WC-1 and WC-2, the trans-acting factors that mediate expression of the circadian clock gene frq, bind at the frq promoter.** Allan Froehlich, Jennifer J Loros, and Jay C Dunlap. Dartmouth Med School, Genetics, Hanover, NH, USA

The frequency (frq)locus is a key component of the *Neurospora crassa* circadian clock. Under constant environmental conditions, frq mRNA and protein oscillate with an approximate 22 hour period; these oscillations are part of an autoregulatory feedback loop in which frq gives rise to two forms of the FRQ protein which then act to repress the levels of the frq transcript (Dunlap, J. Cell 96:271-290, 1999). Evidence gathered to date suggests that resetting of the clock by light occurs through the rapid induction of frq (Crosthwaite et al. Cell 81:1003-1012, 1995). Resection of the frq promoter has uncovered two cis-acting light response elements (LREs), both of which are necessary for light induction of frq to wild type levels. Using a heterologous reporter, both LREs have been shown to be sufficient for light induction. Deletion of the proximal LRE affects phase following a light to dark transfer, but

does not have an effect upon resetting following temperature treatments. Deletion of the distal LRE results in arrhythmia under both light and temperature treatments. The phenotypes of both LRE deletions can be observed at the physiological as well as at the molecular level. White-collar loci products (wc-1 wc-2) have been found to act as global regulators for light perception and as positive components of the circadian clock in Neurospora (Crosthwaite et al. Science 276:763-769, 1997). WC-1 and WC-2 proteins both contain Zn-finger domains with distinct similarity to other transcriptional activators within the GATA factor family. Using electrophoretic mobility shift assays (EMSA), WC-1 and WC-2 have been shown to specifically bind to the frq LREs in two distinct complexes; one complex is present in the light and the other complex is present in the dark. FRQ is not present in either complex. Action spectrum and dose-response data have been generated for changes in binding between the dark and light complexes. These in vitro data correlate well with published in vivo data for light resetting of the Neurospora clock.

454 **Analysis of the *asd-2* gene of *Neurospora crassa.*** Kelly A. Howe, Mary Anne Nelson University of New Mexico, USA

A genetic approach is being used to the investigate the sexual development of *Neurospora crassa*, which is poorly understood at the molecular level. The *asd-2* (ascus development) gene is essential for sexual development; homozygous *asd-2* crosses are blocked shortly after karyogamy. Ascospores are never produced and asci are short and few in number. Small perithecia are formed that lack perithecial beaks. Vegetative growth of *asd-2* mutant strains also appears to be deficient. The ASD-2 protein displays significant homology with the AGO/eIF2C2/Zwille family of proteins. This family of proteins has been implicated in two major processes: involvement in stem cell differentiation and involvement in posttranscriptional gene silencing. One member of this family in particular, the *N. crassa* QDE-2 (quelling deficient) protein, has been shown to be involved in quelling, a particular form of posttranscriptional gene silencing. We are investigating the role of the *asd-2* gene product in cell signalling and/or posttranscriptional gene silencing and the relationship of this role to sexual development.

455 **Initial characterization of genes involved in mushroom development in the basidiomycete *Schizophyllum commune*,as isolated by a two-hybrid screen.** Stephen Horton and Alaap Shah. Department of Biological Sciences, Union College, Schenectady, NY 12308 USA

The molecular mechanisms controlling the development of mushrooms are incompletely understood in basidiomycetous fungi. The gene *FRT1*was originally identified in *Schizophyllumcommune*by its' ability as a transgene to induce fruiting in certain homokaryotic transformation recipients. In an effort to identify new genes involved in fruiting body development, we have utilized *FRT1*as 'bait' in a two-hybrid screen. Frt1p has been localized primarily to the outside of the cell wall, and is therefore likely to be post-translationally modified. We therefore decided to employ the Cytotrap Ras rescue two-hybrid approach to isolate genes encoding interacting proteins, rather than a traditional nuclear-based system. 1.5 X 10^7 transformants were obtained in a screen of two newly-constructed *S. commune*cDNA libraries. 533 initial positives were found, of which 121 passed a second round of screening. Library plasmids were first rescued in *E. coli*,and the cDNA inserts amplified by PCR. These inserts will be sorted into groups by restriction enzyme analysis. Further characterization of representative members of each group will be presented.

456 **Expression of *nsdD* that controls sexual development of *Aspergillus nidulans.*** Kyu-Yong Han, Kap-Hoon Han[1],Seon-Hwa Park, Kwang-Yeop Jahng[2], Keon-Sang Chae[2] and Dong-Min. Han Division of Life Science, Wonkwang University, Iksan 570-749 ; [1]Dept. Microbiology & Molecular Genetics, Oklahoma University Stillwater, OK 74078, USA; [2] Faculty of Biological Science, Chunbuk University, Chunju

The *nsdD* gene predicted to encode a GATA type transcription factor with the type IVb zinc finger DNA binding domain functions in activating sexual development of *A. nidulans*. When the *nsdD* gene was over- expressed, cleistothecia were formed in excess amount even in the presence of 0.6 M KCl that inhibited sexual development of wild type. A lot of suppressors for Delta-nsdD showed the common phenotype similar to that of *nsdD* over-expressed mutant. Northern blot analysis revealed that the expression of *nsdD* was repressed by 0.6M KCl. These results strongly suggest that the inhibition of sexual development by salts was carried out via *nsdD* involved regulatory network. In several allelic mutants of *nsdD* that resulted in non sense mutations and lacked C terminal zinc finger, the transcription level was greatly increased. Also in over-expressed mutants, the transcription under its own promotor was reduced. These results suggest that the expression of *nsdD* is negatively autoregulated. The *nsdD*

expression was greatly reduced in *flbA* deletion mutant, indicating that functional FlbA is required for *nsdD* expression. However, G-alpha, G-Beta or FluG apparently did not have significant relationship with *nsdD* expression. This result suggests that FlbA is not specifically required for signaling of asexual sporulation but also required for that of sexual development, while FluG is specific to the asexual signaling.

457 **The role of hydroperoxidase (laccase II) and alpha-1,3-Glucanase during sexual development of *Aspergillus nidulans*.** Mario Scherer, Huijun Wei and Reinhard Fischer. Max-Planck-Institute for terrestrial Microbiology, Karl-von-Frisch-Str., D-35043 Marburg, Germany.

Sexual development in *Aspergillus nidulans* is an interesting morphogenetic process, which is only poorly understood. We set out to study differentially expressed genes to use them as tools for the isolation of central regulators for development. We isolated laccase II, which was described to be specifically expressed in early developmental structures, such as H lle cells and primordia. The corresponding primary protein sequence revealed that it is a heme-containing hydroperoxidase rather than a laccase. A hydroperoxidase-GFP fusion protein was localized in sexual tissues. The expression pattern revealed an early induction during sexual development and upon carbon starvation. In a non-targeted approach, differentially expressed genes were isolated using the subtractive suppressive hybridisation method (Clontech). One of the identified genes was alpha-1,3-Glucancase, which probably plays a central role in energy supply during sexual differentiation. The gene is specifically induced early during development and can serve to reveal the regulatory circuits. In a targeted approach, a homologue of a transcription factor, Pro1, from *Sordaria macrospora*, which regulates perithecium formation, was isolated from *A. nidulans*. Since overexpression of the gene leads to a misregulation of development, it appears to play a crucial role in *A. nidulans* sexual development. Experiments to analyse a function of this transcription factor for the regulation of the hydroperoxidase and the glucanase are on the way. These studies also include the analysis of the roles of *veA*, *nsdD* and *stuA*, which also trigger sexual development.

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Biochemistry and Secondary Metabolism

458 **Identifying Genes Associated with Ochratoxin A Production in *Aspergillus carbonarius* and *Aspergillus niger*.** Z., Ilic*[1], J.I., Pitt[2], D.A., Carter[1]. [1] Dept of Microbiology, University of Sydney, NSW 2006 [2] Food Science Australia, North Ryde, NSW 2113

Aspergillus carbonarius and *A. niger* belong to the family of black Aspergilli. Black Aspergilli are often found in association with certain commodities, such as dried fruit, grapes and wheat. *A. carbonarius* and to a lesser extent, *A. niger*, are common producers of a chlorinated cyclic polyketide, ochratoxin A (OA). OA is nephrotoxigenic and is a world-wide problem, only recently found to be caused in some cases by the black Aspergilli. Very little is known about the biosynthesis of this secondary metabolite, and it is important to understand OA biosynthesis in order to develop strategies to control this problem. An initial step in understanding toxin production is to assess the genetic differences between toxigenic and non-toxigenic strains. Random Amplification of Polymorphic DNA (RAPD) analysis was used to investigate the genetic background and diversity of *A. niger* and *A. carbonarius* strains of varying toxigenicity. There was no correlation seen between genetic relatedness and degree of to toxigenicity. We have also begun to investigate the genes involved in the OA biosynthetic pathway in *A. carbonarius* and *A. niger*, using both degenerate primer PCR and plasmid mutagenesis. The former uses primers based on conserved regions of the gene for polyketide synthase, an important enzyme in the formation of polyketides such as ochratoxin. For plasmid mutagenesis we are optimising a transformation system for *A. carbonarius* and *A. niger* to introduce the

disruption vector pAN7-1. A simple screen for toxigenicity allows us to identify colonies with insertions into OA biosynthetic genes, thereby allowing the isolation of these genes.

459 **Linkage of molecular markers with trichothecene chemotypes in *Gibberella zeae*.** Nancy J. Alexander[1], Ronald D. Plattner[1], Robert Bowden[2], and John F. Leslie[2]. [1]Mycotoxin Research Unit, National Center for Agricultural Utilization Research, USDA/ARS, Peoria, IL, [2]Dept. of Plant Pathology, Kansas State University, Manhattan, KS

Gibberella zeae (anamorph *Fusarium graminearum*) produces mycotoxins, most notably, deoxynivalenol (DON) and nivalenol (NIV). Most of the strains found in North America produce DON, while NIV-producing strains are more common in Asia. It is not known how the biosynthetic pathway of toxin production differs in the two types of producers. Chemically, NIV differs from DON by having an OH group at the C4 position. In an attempt to distinquish genetic control of toxin formation, we crossed a high DON-producer with a low NIV-producer and analyzed 99 progeny. Progeny were scored for type of toxin produced as well as relative amount of toxin. Southern hybridizations and polymerase chain reaction (pcr) were used to detect polymorphism at the *TRI5* (trichodiene synthase) locus. Results suggest that the type of toxin produced, either NIV or DON, was controlled by a single gene which was completely linked to *TRI5*. The amount of toxin produced, either high or low, was controlled by another single gene which was unlinked to *TRI5* or the toxin type locus. These results should help in our investigations of the ecology of the organism as well as a better understanding of toxin biosynthetic pathways.

460 **Characterization of a Locus Required for Mycotoxin Biosynthesis in Aspergillus spp.** Yongqiang Zhang, Nancy Keller. Department of Plant Pathology and Microbiology Texas A&M University, College Station, TX 77843-2132

Sterigmatocystin (ST) is a carcinogenic polyketide produced in several Aspergillus species including *A. nidulans.* In *A. nidulans,* the ST biosynthesis genes (stc genes) are clustered on chromosome IV. Mutagenesis and subsequent screening have identified 23 mutants that were defective in ST biosynthesis and unlinked to the stc gene cluster. Diploid analysis demonstrates that five of these mutants likely comprise one locus. Furthermore, a genetic segregation study shows that this locus is linked to yA and biA on chromosome I. Two cosmids of the *A. nidulans* trpC library complement the mutated locus. Subcloning and transformation studies identify a 17 kb subclone of these cosmids which can complement the locus. Sequencing data reveals that this 17 kb DNA fragment contains a fatty acid transporter homolog, a translation elongation factor homolog as well as other less defined ORFs. Further investigation to identify the gene required for ST/AF biosynthesis is under way.

461 **Cloning and characterization of a polyketide synthase gene (pksN1) required for synthesis of a perithecial red pigment in *Nectria haematococca.*** Stephane Graziani. Marie-Josée Daboussi. Université Paris Sud, IGM, Orsay France

Most studies of pigment biosynthesis in fungi have focused on dark brown pigments, melanins, shown to be important pathogenic factors for both plant- and animal- pathogens. In contrast to this biosynthesis, little is known about pigment biosynthesis in reddish fungi. In *Nectria haematococca*, mutants affecting the coloration of mycelia and perithecia (hyperproduction of red pigments or albinos) have been obtained through mutagenesis. The analysis of these mutants showed that the lack of pigmentation of the perithecium wall and that of mycelia result from the operation of two distinct biochemical pathways. Some of the pigments formed in the vegetative phase are naphtoquinones. The composition of the pigment found in the wall of perithecium is unknown. Here we reported on the cloning of the gene which is required for the red coloration of perithecia by complementation of the *I4* mutant (white perithecia) by a cosmid library. Subcloning identified a 5 kb DNA fragment able to restore the mutant phenotype. DNA sequencing revealed high degree of homology with polyketide synthase genes. On the basis of the presence of at least the beta-ketoacyl synthase and acyl transferase domains usually present in polyketide synthases and fatty acid synthases, we propose that the *I4* gene encodes the polyketide synthase which initiates red pigment biosynthesis. In addition, we initiated studies on the expression of this gene in order to determine if it is developmentally regulated.

462 **Loline biosynthesis in *Neotyphodium uncinatum*: identifying biosynthetic genes with the use of subtractive hybridization.** Martin J. Spiering[1], Jimmy D. Blankenship[1], Heather H. Wilkinson[2], and Christopher L. Schardl[1]. [1]Department of Plant Pathology, University of Kentucky, Lexington, Kentucky and [2]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, U.S.A.

The insecticidal 1-aminopyrrolizidine alkaloids (*e.g.* N-formyl and N- acetyl loline) are present in the grass meadow fescue (*Lolium pratense*) when infected by the fungal endophyte *Neotyphodium uncinatum*, significantly strengthening the plantÆÆs resistance to insect attack. For isolating key genes involved in loline biosynthesis in *N. uncinatum* we have adjusted culture growth conditions to induction of loline production in mycelium at high levels and to suppression of production, respectively. RNAs isolated from mycelium grown under these conditions were used as æætesterÆÆ (producing) and ædriverÆÆ (suppressed) in subtractive hybridization. Northern analysis showed significant expression of subtracted transcripts only under loline-producing conditions. By using the subtracted cDNAs as probe, several hybridizing (ææ+ÆÆ) clones have been identified from a cDNA library of loline- producing mycelium. Sequence analysis of a ææ+ÆÆ clone indicated a highly significant match with an aspartate kinase, an enzyme involved in the biosynthesis of methionine and threonine. In addition, we have cloned a DNA fragment closely linked to a locus controlling loline production in the endophyte *Epichlo festucae*. Analyses indicated a highly significant similarity to O-acetyl-homoserinesulfhydrylase (OAH), an enzyme likewise involved in the biosynthesis of methionine. The OAH-like gene is highly expressed during loline production in *N. uncinatum*, and was detected only in loline-producing endophytes. These findings suggest a significant role in loline biosynthesis of enzymes normally functioning in methionine biosynthesis, possibly by directing methionine to production of polyamines (e.g. spermidine), which are intermediates in the biosynthesis of related plant pyrrolizidine alkaloids. This work was supported by the U.S. National Science Foundation Integrative Plant Biology Program 9808554.

463 **Antimicrobial and antichagasic activities produced by *Talaromyces flavus*.** Tatiana Pereira da Silva de Freitas, Jairo Kenupp Bastos, Sérgio de Albuquerque, Suraia Said. Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brasil.

Chagas' disease, which is caused by *Trypanosoma cruzi*, attacks about 116 million people in the world and the lack of efficient and nontoxic medicine is a constant concern in tropical countries. In addition, microbial resistance to antibiotics is increasing and many antibiotics are no longer effective. Thus, the search for new antichagasic and antimicrobial drugs is desirable. In the present study *T. flavus* (CCT3138) was inoculated in a two-step culture, the final broth was partiotioned with chloroform and butanol and the extracts were analyzed. The best antimicrobial activity was detected in the chloroform extract obtained from cultures after 192 hours of incubation. The extract from Czapeck medium had the best activity against *Candida albicans* (76% more active than the standard miconazole 4 mg/ml) and against *Micrococcus luteus* (88% more active than the standard penicillin G 0.6075 U/ml). The extract from Takeuchi medium showed the best activity against *Staphylococcus aureus* (200% more active than the standard used, i.e. penicillin G 1.215 U/ml). The chloroform extracts from the Takeuchi and Czapeck media had the same activity against *Escherichia coli* as the standard used (streptomicin 77.7 U/ml). The activity contained in the water extract from the culture broth of Czapeck medium, which was incubated for 96 hours, was responsible for the lysis of 97.58% of trypomastigote forms of *T. cruzi* (red blood cells remained normal). The antichagasic activity was also detected in other extracts but with a lower effect. The standard used to compare the efficiency of the extracts was Gentian violet, which killed 100% of the trypomastigote forms of the parasite. Supported by FAPESP (Proc. n . 00/06847-5).

464 **Comparative sequence analysis of trichothecene biosynthesis gene clusters from DON/NIV chemotypes of *Gibberella zeae.*** Theresa Lee, Daewoong Oh, Hye-Seon Kim, Jeong-Kwan Lee, Sung-Hwan Yun[1], and Yin-Won Lee. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea, [1]Division of Life Sciences, Soonchunhyang University, Asan 336-745, Korea

G. zeae, one of major causative fungi of cereal scab, is chemotaxonomically divided into two groups based on trichothecene production; deoxynivalenol(DON) chemotype and nivalenol(NIV) chemotype. To study genetic diversity of trichothecene production by these two chemotypes frequently found in Korea, the gene cluster required for trichothecene biosynthesis in each chemotype was cloned and sequenced. H-11 was a representative of DON chemotype isolated from corn and 88-1 was a NIV producer from barley. Sequencing analysis revealed a 23kb and a

26kb gene cluster along with unlinked *Tri101* genes from H-11 and 88-1, respectively. Each gene cluster consisted of 8 *Tri* genes and 2 other ORFs (probably *Tri9* and *Tri10*) with the same order and transcriptional directions as that of *Fusarium sporotrichioides*. Comparative sequence analysis showed that all the genes except *Tri7* were very conserved among H-11 and 88-1, ranging from 87.8 to 95.9% at nucleotide level and from 81.8 to 97.3% at amino acid level. However, *Tri7* was strikingly different showing 78.8% similarity at nucleotide and 53.9% at amino acid level. Sequence analysis of *Tri7* from other DON and NIV producers revealed that all the *Tri7* ORFs from DON chemotypes tested carried several mutations and an intron containing different numbers of a 11bp-tandem repeat while none of the NIV chemotype tested had the repeat or mutations. This result indicates that *Tri7* may be a key enzyme for differentiation of DON and NIV in the trichothecene biosynthetic pathway. Further study to determine a function of *Tri7* in DON/NIV chemotypes is in progress.

465 **Genetic manipulation of *Gibberella zeae* for increasing outcross** Jeong-Kwan Lee, Sung-Hwan Yun[1], Theresa Lee, and Yin-Won Lee. School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea, [1]Division of Life Sciences, Soonchunhyang University, Asan 336-745, Korea

G. zeae, a self-fertile (homothallic) ascomycete is an important plant pathogen causing head blight of wheat and barley, and stalk or ear rot of maize. In addition, the fungus produces mycotoxins including trichothecenes and zearalenone on diseased crops so it has been a potential threat to human and animal health. However, homothallism of this fungus has been an obstacle to genetic analysis of pathogenicity as well as mycotoxin production of *G. zeae*. To circumvent this barrier, several *G. zeae* isolates producing either deoxynivalenol (DON) or nivalenol (NIV) were genetically manipulated to be used as parents of sexual crosses in this study. First, each representative of two chemotypes was transformed with either of two drug resistance genes ($HygB^R$ for resistance to hygromycin B and Gen^R for geneticin), creating transformants with either DON;$HygB^R$ or NIV;Gen^R. A significant number of sexual progeny showing resistance to both drugs were easily obtained from a cross between two different transformants and the progeny segregated independently for the mycotoxin chemotype and drug resistance. Second, a mating type gene (*MAT-2*) of a DON producer, which carries two opposite *MAT* genes (*MAT-1* and *MAT-2*) in a single nucleus, was deleted and replaced with a vector carrying a green fluorescent protein gene. The resulting *MAT-2*-deleted strain was self-sterile, suggesting that both *MAT* genes are required for homothallism of *G. zeae*. A cross between this *MAT-2*-deleted DON producer and a homothallic NIV producer produced fertile recombinant perithecia on the mating plate although the fertility was lower than that of self of wild type. Ascospores from those perithecia segregated independently for green fluorescence and mycotoxin chemotype, suggesting normal meiosis occurred in this cross. Thus, use of drug resistance genes and *MAT* gene manipulation provided simple and efficient ways to obtain sexual recombinants of *G. zeae*, which allows genetic analysis of this fungus.

466 **Enhancement of antimicrobial activity produced by *Aspergillus fumigatus*.** Niege Araçari Jacometti Cardoso Furtado, Suraia Said, Izabel Yoko Ito, Jairo Kenupp Bastos. Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brasil.

In the course of a screening program for new antibiotic producers, a strain of *Aspergillus fumigatus* was isolated from Brazilian soil samples (Pantanal, MS). This fungus was first grown on seed medium to achieve biomass and after 24 hours it was transferred to a production medium. A pool of living or autoclaved microorganisms was added or not to the fungus culture on the second day in the production medium at the ratio of 1:6 to increase antibiotic production. After different times the culture broths were separated from mycelium and extracted with chloroform and butanol. Extracts were analyzed by HPLC and submitted to antimicrobial activity evaluation by agar diffusion assay and bioautography. There was a 55% increase in activity against *Staphylococcus aureus*, a 63% increase against *Candida albicans* and more than 100% against *Micrococcus luteus* in chloroform extracts from the broth of cultures in which the pool of living or autoclaved microorganisms was present. The chromatographic profiles of chloroform extracts from the two culture conditions were different. By bioautography two additional active compounds were detected in chloroform extract from the broth of cultures grown in the presence of pooled microorganisms and 1,3,5-trimethoxybenzene and 2,5-dimethoxyphenol were identified in this extract. Supported by FAPESP (Proc. n . 99/09850-8).

467 **Localisation of ACV synthetase of *Aspergillus nidulans.*** William Vousden and Geoffrey Turner. Department of Molecular Biology and Biotechnology, Krebs Institute for Biomolecular Research, Western Bank, Sheffield, UK

The biosynthetic pathway for penicillin is now well understood from extensive studies in both *Penicillium chrysogenum* and *Aspergillus nidulans*. There is also evidence for compartmentalisation of the pathway, since it has been shown that acyl transferase, the enzyme responsible for the final step in biosynthesis, is located in microbodies. Localisation of L-aminoadipyl-L-cysteinyl-D-valine synthetase (ACVS), the peptide synthetase which carries out the first step in the pathway, proved more difficult using available antibodies, though there had been reports that it might be membrane associated, possible within the vacuole. We have used a GFP fusion construct in an attempt to resolve this problem. GFP was fused to the 5' end of the ACVS, since previous work had indicated that loss of some of the N-terminal amino acids did not prevent penicillin biosynthesis. This fusion gene was placed under the control of the strong, regulatable *alcA* promoter, and introduced into *A. nidulans* using a gene replacement technique. A single copy transformant was analysed for penicillin production and GFP localisation within the mycelium. The strain was still able to produce penicillin from the GFP-ACVS fusion, though at a lower yield than wild-type, indicating partial functioning of the gene fusion. Fluorescence microscopy revealed that the GFP was found throughout the mycelium, but appeared to be excluded from the vacuoles. It appears that vacuolar localisation of ACVS is not required for penicillin biosynthesis.

468 **Functional analysis and expression studies of the gibberellin biosynthesis genes in *Gibberella fujikuroi*.** Bettina Tudzynski, Martina Mihlan and Jessica Schulte. Westfälische Wilhelms-Universität Münster, Institut für Botanik, Schlossgarten 3, 48149 Münster, Germany

Gibberella fujikuroi produces large amounts of gibberellins under conditions of nitrogen limitation. Recent studies have shown that at least 6 genes of the gibberellin (GA)-biosynthetic pathway are clustered in chromosome 4 in the *G. fujikuroi* genome; these genes encode the bifunctional *ent*-copalyl diphosphate synthase/*ent*-kaurene synthase (*cps/ks*), a GA-specific geranylgeranyl diphosphate synthase (*ggs2*) and four cytochrome P450 monooxygenases. (Tudzynski et al. 1998, Tudzynski and Hölter,1998). To study the function of several genes of this cluster, we used a gene replacement approach followed by GC-MS and HPLC analysis of the mutant culture fluids as well as feeding experiments. Since the biosynthesis of fungal gibberellins includes at least 13 enzymatic steps, we expected some of the biosynthesis genes to have a multifunctional character. To find out the pathway steps catalyzed by each of the P450 monooxygenases, we transformed the single genes into a *G. fujikuroi* GA-defective mutant which lacks the entire GA gene cluster due to a large deletion on chromosome 4 (Linnemannstöns et al. 1999). The single genes are highly expressed in this mutant strain, clearly demonstrating that the regulation factors needed for their expression are not located within the gene cluster. Using this procedure we have identified the multifunctional character of both P450-1 and *P450-4*. Because the gibberellin biosynthesis is regulated by ammonium, we cloned several genes which are involved in nitrogen regulation, such as the general nitrogen regulators, *areA* and *nmr*, as well as *gs* and *gdh1* encoding a glutamine synthase and a NAD-dependent gluatamate dehydrogenase. Gene replacement of *areA* led to a significant reduction of gibberellin formation by repressing the expression of the GA-pathway genes. Complementation of the *areA*-deficient mutant with the *areA* wild-type copy completely restored the gibberellin production, which suggests that the positive acting regulatory protein AREA directly controls the transcription of the pathway (Tudzynski et al 1999). References B. Tudzynski, H. Kawaide, and Y. Kamiya (1998). Curr. Genetics, 34: 234-240. B. Tudzynski and K. Hölter (1998). Fungal Genetics and Biology, 25: 157-170. B. Tudzynski, V. Homann, B. Feng, and G.A. Marzluf (1999). MGG, 261: 106-114. P. Linnemannstöns, T. Vo , P. Hedden, P. Gaskin and B. Tudzynski (1999). Appl. Env. Microbiol. 65: 2558-2564.

469 **Isolation and characterization of a second gene in the pyridoxine synthesis pathway of fungi, plants, archaebacteria and a subset of eubacteria.** Marilyn Ehrenshaft and Margaret E. Daub, Dept. of Plant Pathology, NCSU, Raleigh, NC, 27695

Recent work in our laboratory uncovered definitive evidence that the majority of the biological world uses a pathway for pyridoxine (vitamin B6) synthesis that diverges from that of the well-characterized *Escherichia coli* pathway. The *Cercospora nicotianae PDX1* gene was originally isolated as part of a project to genetically characterize *C. nicotianae* resistance to its own endogenous photosensitizer, cercosporin, and to other photosensitizers of diverse structure and solubility. *PDX1* homologues belong to one of the most highly conserved protein families yet identified, and are found in fungi, plants, and archaebacteria, as well as in many eubacteria that do not encode homologues to the *E. coli* pyridoxine genes. Homologues to the gene we are now calling *PDX2* were first noted because they were consistently found in the same subset of organisms that encoded *PDX1* homologues. In some organisms they were found in the same operon (*Bacillus subtilis* and *Haemophilus influenzae*) or within 500

bp of each other (yeast). We have isolated the *C. nicotianae PDX2* gene using a degenerate primer strategy. We provide direct evidence this gene is involved in pyridoxine synthesis via complementation of pyridoxine auxotrophs and also by our production of a pyridoxine auxotroph via gene disruption of *pdx2*. We show that PDX2 proteins are less well conserved than their PDX1 counterparts but contain several conserved protein motifs. We also present evidence to support the hypothesis that the enzymatic step catalyzed by the PDX1 protein precedes that of the step catalyzed by the PDX2 protein.

470 **New mutants of the carotenoid pathway of *Neurospora crassa.*** Javier Avalos[1], Loubna Youssar[1], Nabil Arrach[1], and Tom J. Schmidhauser[2]. [1]Departamento de Genetica, Universidad de Sevilla, Spain [2]Department of Biology, University of Louisiana at Lafayette, LA, USA

Three novel kinds of carotenoid mutants of *N. crassa* have been identified. Two of them were isolated taking advantage of the accumulation of a higher proportion of final products of the carotenoid pathway upon photoinduction at low temperature. Using illumination at 6 C as screening conditions, two types of pale reddish mutants have been isolated. Two of them lack neurosporaxanthin and accumulate a mixture enriched in at least one polar carotenoid. Both mutants are complemented by *al-2* and their mutant *al-2* alelles have been sequenced. They are presumably affected in the cyclase activity of Al-2, as deduced from the phenotype and the location of the mutations in the cyclase domain of similar genes in other fungi. The chemical nature of the carotenoids accumulated by these mutants is under investigation. Two mutants of a second type have a different reddish pigmentation, lack neurosporaxanthin and are not complemented by *al-2*. Experiments to determine their biochemical phenotype are under way. Extensive UV mutagenesis was carried out in an attempt to isolate mutants pigmented in the dark. No mutants could be identified out of 500,000 survivors from a wild type strain, but two orange mutants were isolated out of 20,000 from an *ovc* mutant. The *ovc*phenotype is characterized by an increased carotenoid biosynthesis in the light. The two mutants, called *lic* (light independent carotenogenesis) accumulate carotenoids in the dark and show colonial growth and enhanced conidiation. Phenotypic characterization of these mutants is in progress.

471 **Isolation and characterization of two key regulatory enzyme genes involved in isoprenoid metabolic biosynthesis of *Aspergillus nidulans***. Guangyi Wang and Jay D. Keasling. Chemical Engineering, U. C. Berkeley, Berkeley, CA

Isoprenoids are a fascinating family of compounds derived from mevalonate. Fungal isoprenoids comprise a structurally diverse group of primary and secondary metabolites, which range from fungal plant hormones, to mycotoxin, to antimicrobial agents, to pharmaceutically important antibiotics. In many cases, the low production level of the isoprenoid of interest in the native source is a major limiting factor for practical extract and purification before use. 3-hydroxy-3-methylgutaryl-CoA reductase (HMGR) and isopentenyl pyrophosphate (IPP) isomerase are two key regulatory enzymes in the isoprenoid metabolic pathway. HMGR catalyzes the reduction of HMG-CoA to mevalonate and IPP isomerase converts IPP to dimethylallyl diphosphate (DMADP), a reversible reaction. Over-expression of the two enzymes has been shown to dramatically increase carotenoid production of interest in several systems. In order to engineer metabolic biosynthesis of isoprenoids in *Aspergillus nidulans*, genomic fragments of HMGR and IPP isomerase have been isolated from *A. nidulans* by homology. Sequence analysis revealed high homology of HMGR and IPP isomerase gene fragments from *A. nidulans* with the HMGR and IPP isomerase genes from other organisms. The genomic DNA fragments have been used as probes to isolate full-length genes from a cosmid genomic library of *A. nidulans*.

472 **Mapping a locus involved in loline alkaloid expression in the fungal endophyte *Epichlo festucae.*** Gang, Liu[1], Mayfield, Coreline T.[1], Schardl, Christopher L[2]. And Wilkinson, Heather H[1]. [1] Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas and [2]Department of Plant Pathology, University of Kentucky, Lexington, Kentucky

Loline alkaloids (saturated 1-aminopyrrolizidine alkaloids with an oxygen bridge) are fungal secondary metabolites often present in grasses symbiotic with endophytes in the genera Epichlo and Neotyphodium. Endophytes producing loline alkaloids provide grass hosts with enhanced protection from herbivory, drought and pathogens. In an earlier study, segregation analysis supported a single locus contributing to a naturally occurring lolines expression polymorphism in Epichlo festucae (Lol+ vs. Lol-). Identification and cloning of an AFLP marker tightly linked to the Lol+ phenotype has permitted map based cloning of at least a portion of that locus. A 6,144 clone bacterial

artificial chromosome (BAC) genomic library of a Lol+ E. festucae parent was constructed. Current analysis centers around one 115 kb BAC clone containing the AFLP marker. End sequencing of this and overlapping BACs provided additional markers (0.2 to 4.5 kb in length). Southern analysis showed that markers identified at one end of the BAC are present in both the Lol+ and the Lol- parent. Markers located closer to the opposite end of the BAC, from 85 to 115 kb away from the shared region, only hybridize to DNA from the Lol+ parent. Preliminary sequence analysis indicated genes in this unique region related to those for ornithine decarboxylase (ODC) and an O-acetylhomoserinesulfhydrylase/thio-lyase (OAH). ODC catalyzes putrescine synthesis and OAH can serve in a methionine synthesis pathway. Both putrescine and methionine are precursors of spermidine, so it is of interest that, based on their structure, loline alkaloids have previously been suggested to be spermidine derivatives. Our data support a relationship between lolines and polyamines.

473 **Analysis of an ergovaline-deficient *Neotyphodium* endophyte resulting from a peptide synthetase gene knockout.** R. D. Johnson[1], P. Damrongkool[1], J. Wang[2], C. L. Schardl[2] and D. G. Panaccione[1]. 1 West Virginia University, Morgantown; 2 University of Kentucky, Lexington.

Toxic ergopeptines are produced by several fungi, including the ergot fungus *Claviceps purpurea* and the tall fescue endophyte *Neotyphodium coenophialum*. Ingestion of ergopeptines, particularly ergovaline, by livestock results in poor performance and toxicoses. The biosynthetic pathways involved in the production of these compounds has begun to be elucidated and appears to require the activity of two peptide synthetases. Several lines of evidence, from *C. purpurea*, indicate that a gene named Cp605 encodes one of these peptide synthetases and we have shown that this gene has homologues among ergopeptine producing endophytes of the Clavicipitaceae. We have knocked out a Cp605 homologue (*LPS1*) in *Neotyphodium* sp. strain Lp1 by gene replacement and have analysed its effect on ergovaline production during the association with perennial ryegrass. HPLC analysis showed that grass associations containing the knockout strain of Lp1 failed to produce ergovaline. However, competitive PCR (using primers to an internal fragment of *LPS1*) on grass associations containing the gene knockout transformant, ectopic transformants and wild type Lp1, established that fungal biomass was not effected in planta. Interestingly, absence of ergovaline production appears to be associated with the presence of a novel HPLC peak of grass origin.

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474 **Genetic analysis of four genes in the paxilline biosynthesis gene cluster from *Penicillium paxilli***. Lisa McMillan, Carolyn Young and Barry Scott. Institute of Molecular BioSciences, College of Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand.

Indole-diterpenes are a family of potent tremorgenic mammalian mycotoxins and known inhibitors of maxi-K ion channels. While the chemical complexity of indole-diterpenes are well documented, very little is known about the nature of their biosynthesis or the biochemical intermediates. We recently cloned a gene cluster for the biosynthesis of paxilline, an indole diterpene synthesized by *Penicillium paxilli* and a proposed intermediate in the biosynthesis of other indole-diterpenes (Young et al. 2001). The cluster is predicted to lie within a 50 kb region on chromosome Va and to contain 17 genes, including a geranylgeranyl pyrophosphate (GGPP) synthase (*paxG*), two FAD-dependent monooxygenases (*paxM* and *paxN*), and two cytochrome P450 monooxygenases (*paxP* and *paxQ*) (Young et al. 1998, 2001). The gene structures of four of these genes, *paxG*, *paxM*, *paxP* and *paxQ*, were further characterised by RT-PCR and RACE. Gene replacements using homologous recombination were performed and the chemical phenotypes determined by TLC and HPLC. The four gene replacements were all paxilline negative. Deletion of *paxG* and *paxM* have no detectable intermediates suggesting an early role for the enzymes encoded by these genes, while *paxP* and *paxQ* gene replacements accumulate the intermediate paspaline and 13-desoxypaxilline respectively. This is the first indole-diterpene gene cluster to be cloned and characterised.

475 **Molecular cloning of indole-diterpene biosynthetic genes from *Neotyphodium lolii***.Carolyn A. Young and Barry Scott. Massey University, IMBS, Palmerston Nth, New Zealand

Lolitrem B is well documented as the cause of ryegrass staggers in animals grazing on *Neotyphodium lolii*-infected pastures. Although much is known about how lolitrem B is produced, the genes and proteins responsible have not been isolated. Working with *Penicillium paxilli*we have recently identified a biosynthetic gene cluster for producing

paxilline, a proposed intermediate of lolitrem B. This cluster is comprised of 17 genes, including *paxG*,a geranylgeranyl pyrophosphate synthase (GGPPS), *paxM*and *N*, two monooxygenases, and *paxP*and *Q*,two P450 monooxygenases. Two independent copies of GGPPS are present in the *P. paxilli*genome where *paxG*is specifically responsible for paxilline production and the second, *ggs1*,is required for primary isoprenoid biosynthesis.

To target the *N. lolii*paxilline homologues, degenerate PCR was used with primers designed to *paxG*,the most highly conserved gene in the paxilline pathway. Two 220 bp fragments, CY28 and CY29, were isolated and shown to encode two distinct GGPPS genes. Southern analysis of *Epichlo typhina*and three *N. lolii*strains showed CY29 sequences are present in all species, while CY28 sequences are seen only in *N. lolii*strains indicating the former is a homologue of *ggs1*and the latter is a homologue of *paxG*.Sequence analysis of a lambda clone containing CY28 revealed three genes, *lolG*, encoding a GGPPS, *lolM*, a monooxygenase and *lolP*, a P450 monooxygenase. All three genes share strong similarity to those found in the paxilline gene cluster. Further analysis will confirm the role of these genes in lolitrem B production.

476 **Independent expression of AGK and AGPR in *Neurospora crassa*.** Mazen Karaman and Richard L. Weiss. Department of Chemistry and Biochemistry University of California, Los Angeles

The complex *arg-6* locus in *Neurospora crassa* encodes a polyprotein precursor for two early mitochondrial arginine biosynthetic enzymes, acetylglutamate kinase (AGK) and acetylglutamyl phosphate reductase (AGPR). This polyprotein is processed into two mature proteins as it is translocated into the mitochondria. Processing involves cleavage of the polyprotein at three different sites. Cleavage of the first site, upstream of the proximal AGK, removes the mitochondrial targeting sequence. The two other sites are upstream of the distal AGPR and cleavage removes a 20-amino acid region connecting both enzymes. The presence of a multi-protein coding gene is rare in eukaryotes; however, *arg-6* homologues have persisted in polyprotein form in *N. crassa* and three other fungi. This work examines the biological significance of this unusual gene/protein structure by investigating the effects of encoding AGK and AGPR on separate genes. Genes expressing AGK and AGPR independently of each other have been introduced into an *arg-6* null strain. The independently expressed proteins can rescue the auxotrophic phenotype of the null mutant with little effect on growth rate or mitochondrial targeting of the proteins. AGK, AGPR, and arginine levels, however, appear to be compromised in these transformants. The results indicate that the polyprotein structure is important for normal expression of AGK and AGPR which, in part, is needed to maintain normal levels of mycelial arginine.

477 **Effect of the nap mutation on growth, carotenogenesis, and respiratory chain in *Neurospora crassa*.** Tatiana A. Belozerskaya[1], Yuri V. Ershov[2], Natalia N. Gessler[2], Elena P Isakova[3], Eugene I Shurubor[3], Ludmila V. Gorpenko[3], and Renata A Zvyagilskaya[3]. [1]A.N.Bach Inst. Biochem., Russian Acad. Sci., Lab. of Evol. Biochem., Moscow, Russia. [2]A.N.Bach Inst.Biochem., Lab.vitamins, Moscow Russia. [3]A.N.Bach Inst.Biochem., Lab.oxid.phosph., Moscow Russia

Down-regulation of metabolism due to reduced solute flux in the transport mutant *nap* brought about the slowed growth without sacrificing general homeostasis (carotenoid levels and biosynthetic rate). The mutant strain was found to have an elevated level of carotenoids both in dark and in the light, and higher intracellular ATP content. A comparative study has been made of mitochondria isolated from wild-type *Neurospora* and from *nap*. Oxidation of succinate and exogenous NADH by mitochondria from wild-type cells, but not by mutant cells, at the stationary growth phase was accompanied by cyanide-resistant respiration (16 to 32% of the total respiration). By contrast, in *nap* cells, the electron flux invariantly (at all growth stages examined) was mediated by cytochrome oxidase with the minor contribution of the nonphosphorylating alternate oxidase. An essential difference between mitochondria from wild-type and *nap* was also revealed during respiration with pyruvate (plus malate). Thus, higher intracellular ATP content in the transport-defective mutant might serve to regulate carotenoid pathway in *nap*. Further characterization of the antioxidant systems in the two strains will be presented. The work was supported by the Russian Foundation for Basic Research grant # 98-04-48382.

478 **ABC transporters in *Fusarium proliferatum*.** Cees Waalwijk, Thamara Hesselink and Gert Kema. Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands.

Among the toxigenic species of the genus *Fusarium*, *F.proliferatum* is known to produce the largest spectrum of mycotoxins, including beauvericin, fumonisin, fusaproliferin and moniliformin. These secondary metabolites are produced within the cytoplasm and must be exported to the exterior to excert their toxic effects. ABC transporters are likely candidates for the secretion of these compounds. In *Saccharomyces cerevisiae*, analyses of the entire genome sequence has revealed the presence of 29 ABC-transporter genes, that have been grouped into 6 classes based on the topology of the deduced proteins. Degenerate primers were designed that are based on the sequences from the genes in the two largest classes, and these primers were used to amplify PCR fragments from several toxigenic *Fusarium* spp. Sequence analyses revealed that these fragments show homology to different genes. Subsequently, a BAC library of *F. proliferatum* (constructed in pBeloBAC 11 and consisting of 10 genome equivalents with 50kb-100kb inserts was screened by PCR. Several positive clones were identified and sequence analysis revealed complete genomic copies of two ABC transporters, *Fp*ABC1 and *Fp*ABC2, with strong homologies to the so-called PDR- and MDR-like ABC transporters, respectively. Northern blot analysis was performed to study the expression of ABC1and ABC2 under different conditions including those inducive for mycotoxin production as well as under conditions of fungicide stress. Several ABC-like gene homologs derived from published EST sequences were also included in these experiments.

479 **The Antifungal Protein from *Aspergillus giganteus* causes permeabilization of fungal membrane**s. T. Theis, M. Wedde, U. Stahl. Department of Microbiology and Genetics, University of Technology Berlin, 13355 Berlin, Germany

Aspergillus giganteus is known to secrete two small basic proteins, the ribonuclease alpha-Sarcin and the Antifungal Protein (AFP). AFP inhibits the growth of several filamentous fungi mainly from the genera *Aspergillus* and *Fusarium* . The minimal inhibitory concentration (MIC) ranges from 0.1 microgram/ml for *Fusarium oxysporum* up to 200 microgram/ml for *Aspergillus nidulans* (1). The protein has no effect on the growth of yeast or bacteria. AFP shares some structural features with other antimicrobial proteins whose inhibitory effect is often due to an interaction with membranes. A similar mode of action was proposed for the AFP. Previous studies revealed that after treating AFP-sensitive fungi with AFP the protein is localized at the hyphal membrane (1). A more detailed analysis of the mode of action was carried out by using an assay based on the uptake of SYTOX-Green (2). Current data from this assay indicates that incubation of AFP-sensitive fungi with the protein resulted in membrane permeabilization, measured as SYTOX-Green uptake. The concentration of the protein and time span of incubation are also important factors for AFP- induced permeabilization. Membrane permeabilization could readily be detected after 5 min. of incubation. The results from the uptake assay correspond very well to those of the localization experiments. It is therefore reasonable to conclude that the growth inhibitory effect of AFP is due to permeabilization of fungal membranes.

(1): T. Theis et al. (1999) ECFG5 Abstract Book: IV-35 (2): K. Thevissen et al. (1999) Appl. Environ. Microbiol. 65: 5451-5458

480 **Interaction of propionate, acetate and glucose metabolism in *Aspergillus nidulans.*** Matthias Brock and Wolfgang Buckel. Philipps-Universitat Marburg, Karl-von-Frisch-Strasse, 35032 Marburg, Germany

Filamentous fungi are able to grow on propionate as sole carbon and energy source *via* the methylcitrate cycle. However, propionate inhibits growth of *Aspergillus nidulans* on glucose but not on acetate. The growth is inhibited even stronger in a methylcitrate synthase deletion strain. Methylcitrate synthase is a key enzyme of the methylcitrate cycle and catalyses the condensation reaction of propionyl-CoA and oxaloacetate to form methylcitrate. During growth on glucose/propionate medium high amounts of propionyl-CoA are accumulated in the mutant strain. Under these conditions succinyl-CoA synthetase is inhibited strongly. At 0.2 mM acetyl-CoA and 0.4 mM propionyl-CoA the *in vitro* activity of succinyl-CoA synthetase is 95% inhibited, whereas citrate synthase and 2-oxoglutarate dehydrogenase are not affected. A second target for inhibition of glucose metabolism is the pyruvate dehydrogenase complex. Growth of wildtype and mutant *A. nidulans* strains on glucose/propionate show an excretion of pyruvate in a millimolar range, probably due to the inhibition of pyruvate dehydrogenase. Addition of acetate to glucose/propionate medium showed a restoring effect on growth, implicating a shunt of building blocks from acetate into the impaired citrate cycle. Despite the fact that acetate metabolism is CreA regulated, significant amounts of acetate are taken up during growth on glucose/propionate/acetate medium. The uptake of acetate is not observed in *A. nidulans* wildtype strains during growth on glucose/acetate medium. These observations implicate that

cometabolism of propionate interferes with normal metabolic regulation, probably by simulating starvation through inhibition of enzymes from other metabolic pathways.

481 **Characterization of progesterone receptors and identifiaction of some of their agonist/antagonists in *Rhizopus nigricans.*** Helena Lenasi, University of Ljubljana, Faculty of Medicine, Institute of Biochemistry, Ljubljana, Slovenia.

It is known that steroid hydroxylating enzyme system containing cytochrome P450 as a terminal oxydase could be induced in filamentous fungus *Rhizopus nigricans*. Enzymes could be induced by progesterone and different steroidal inducers, most probably with the purpose of rendering fungitoxic substrates into less toxic hydroxylated products. During our investigation of the mechanism of the induction process by progesterone we revealed specific progesterone binding molecules in the cytosol and in the plasma membrane. The structure/affinity relationship of cytosolic binding sites and various ligands was examined by cross-displacement studies using different labeled and nonlabeled steroids. Binding components exhibited the highest affinity for progesterone, followed by R5020 and testosterone, whereas dexamethasone and estradiol-17beta showed no binding ability. Mammalian progesterone antagonists onapristone and mifepristone were not able to displace labeled progesterone indicating an important structural difference between progesterone receptors in mammals and lower eucaryotic microorganism. In search for the possible other antagonists of progesterone we examined the action of alpha-naphtoflavone which was not able to induce hydroxylating enzymes in *R. nigricans*. The displacement of labeled progesterone by alpha-naphtoflavone was as efficient as by nonlabeled progesterone. Moreover, alpha-naphtoflavone prevented in a dose-dependent manner the induction of enzymes by progesterone. These results strongly suggest the antagonistic action of alpha-naphtoflavone as well as the involvement of soluble progesterone receptors in the enzyme-induction by progesterone in *R. nigricans*. Further potential inducers/ligands originating from natural fungal growing environment are under investigation.

482 **Dimethylallyltryptophan synthase genes associated with endophyte ergot alkaloids.** Christopher L. Schardl and Jinghong Wang. University of Kentucky, Plant Pathology, Lexington, Kentucky, USA

Ergot alkaloids produced by some mutualistic, seed-borne fungal symbionts of grasses (the *Neotyphodium* spp. endophytes) are associated with livestock toxicosis. A potential solution involves disrupting the gene for the first commitment step in ergot alkaloid biosynthesis. We cloned the dimethylallyltryptophan synthase (DMAT synthase) gene *(dmaW)* from *Claviceps fusiformis, C. purpurea* and *Balansia obtecta*, and confirmed identities of the gene products. We then compared predicted amino acid sequences, derived degenerate primer sets for PCR, and cloned four *dmaW* homologs from three *Neotyphodium* spp. The deduced amino acid sequences shared 60-74% identity with DMAT synthases from *Claviceps* spp. and *B. obtecta*. Transcripts of *dmaW* were detected from cDNA libraries of tall fescue symbiotic with *N. coenophialum*, but not from *N. coenophialum* grown on potato dextrose agar nor from non-symbiotic tall fescue. Since *N. coenophialum* produces alkaloids in planta but not in uninduced culture, this result is consistent with *dmaW* encoding the determinant step in the pathway. The presence or absence of *dmaW* homology in 19 endophyte species was analyzed by Southern blot and PCR. All ergot alkaloid producers investigated contained *dmaW* homology and most ergot alkaloid non-producers lacked such homology, further indicating that *dmaW* is dedicated to biosynthesis of ergot alkaloids. Therefore, *dmaW* genes of grass endophytes are appropriate targets for knock-outs to generate ergot alkaloid non-producing endophytes to be incorporated into forage grass breeding lines and cultivars. USDA-NRI 98-35303- 6663

483 **A genomic and biochemical approach to trichothecene diversity in *Fusarium sporotrichioides* and *Fusarium graminearum.*** Daren W. Brown, Susan P. McCormick, Nancy J. Alexander, Robert H. Proctor, and Anne E. Desjardins. Mycotoxin Research Unit, USDA/ARS, National Center for Agricultural Utilization Research, Peoria, IL 61604

The trichothecenes T-2 toxin and deoxynivalenol (DON) are fungal natural products that are toxic to both animals and plants. Their importance in the pathogenicity of *Fusarium* spp. on crop plants has inspired efforts to understand the genetic and biochemical mechanisms leading to trichothecene synthesis. In order to better understand T-2 toxin biosynthesis by *Fusarium sporotrichioides* and DON biosynthesis by *F. graminearum*, we compared the nucleotide sequence of the 23-kb core trichothecene gene cluster from each organism. This comparative genomics allowed us to predict proteins encoded by two trichothecene genes, *TRI9* and *TRI10*, that had not previously been described

from either *Fusarium* species. Differences in gene structure were also correlated with differences in the types of trichothecenes the two species produce. Gene disruption experiments showed that *F. sporotrichioides TRI7* (*FsTRI7*) is required for acetylation of the oxygen on C-4 of T-2 toxin. Sequence analysis indicated that *F. graminearum TRI7* (*FgTRI7*) is non-functional. This is consistent with the fact that the *FgTRI7* product is not required for DON synthesis in *F. graminearum* because C-4 is not oxygenated.

484 **Influence of Corn Silk Extracts and Flavonoids on Differentiation and Mycotoxin Synthesis by *Aspergillus nidulans.*** Carlos Bucio, Hugo A. Luna and Doralinda Guzman-de-Pena. Unidad Irapuato. CINVESTAV-IPN, Micotoxin Laboratory, Irapuato, Guanajuato, Mexico

Several reports have indicated that plant substances can modifyd mycotoxin synthesis on Aspergilli suggesting a chemical communication between plant and fungus. However, information about the effect of specific plant regulators and flavonoids present in silk corn, on the differentiation process of the fungi and its capacity to produce aflatoxins, has not been evaluated. In this work we study the effect of these compounds on differentiation and synthesis of Sterigmatocystin by Aspergillus nidulans. One (naphthalene-acetic acid) out of eight growth regulators showed an inhibitory effect on both processes. The ethanolic silk extract showed a similar effect. Silk tissues on the contrary showed an increase on sporulation and mycotoxin synthesis.Studies on the effect of these substances on the expression of genes involved in sporulation (flbA and brlA1) and mycotoxin synthesis (aflR) are in progress.

485 **Nonribosomal peptide synthetases of *Cochliobolus heterostrophus*.** Bee-Na Lee, Olen C. Yoder and B. Gillian Turgeon. Novartis Agricultural Discovery Institute, San Diego, California, U.S.A.

Small molecules produced by fungi or bacteria are key candidates as effectors of the plant microbe interaction. Members of one class of these are peptides synthesized nonribosomally by multifunctional enzymes called nonribosomal peptide synthetases (NRPSs). The genes encoding these enzymes are large, ranging from 7 to 50 kb. All NRPSs exhibit a similar modular organization. Each module contains six core signature sequences in separate domains. One domain (5 core signatures) is responsible for amino acid activation (adenylation) and the other (core 6) is responsible for polymerization of amino acid residues. Recently, a conserved peptide synthetase (CPS1) was characterized from *Cochliobolus heterostrophus*. Deletion of CPS1 reduces virulence on corn. To further explore the role of NRPSs in fungal virulence, we are analyzing all NRPS-encoding genes in the *C. heterostrophus* genome. For functional analysis, primers are designed to generate constructs that delete the conserved core sequences (core 1-core 6) of at least one module. Deletion constructs are linearized and used for fungal transformation. Each transformant carrying a targeted deletion is verified by PCR and then screened on plants for changes in virulence compared to wild type.

486 **Identification of differentially expressed genes during aflatoxin biosynthesis in *Aspergillus flavus*.** Greg R. OBrian and Gary A. Payne. Department of Plant Pathology, North Carolina State University, Raleigh NC 27695-7616.

Aflatoxins are polyketide derived secondary metabolites whose biosynthesis can be induced by simple sugars. We are interested in identifying those genes differentially expressed during aflatoxin biosynthesis and profiling their expression during aflatoxin biosynthesis. Candidate genes for study were identified by hybridizing an array of 100,000 *Aspergillus flavus* cDNA clones with probes made from mRNA collected from cultures of the fungus in the log phase of aflatoxin production. A subset of clones expressed primarily during aflatoxin biosynthesis was chosen for sequence analysis. Quality EST sequences (100 base pairs or greater with at least phred 20) were obtained for over 2200 clones. Sequence analysis revealed the presence of over 600 unique clones. Many of the unique ESTs showed no homology to sequences in the public databases. Among ESTs with homology to sequences with known functions are those coding for signal transduction pathways, secondary metabolism, glucose regulation, cell wall biosynthesis, and cell cycle control. We have developed a microarray containing replicates of our unique clone set and are in the process of further analyzing these clones to establish a gene expression profile during aflatoxin biosynthesis.

487 **Production and use of expanded DNA microarrays for characterization of trichothecene production.** Andrew G. Tag[1], Andrew W. Peplow[1], Tzung-Fu Hsieh[2], Terry L. Thomas[2], and Marian N. Beremand[1]. [1]Texas

A&M University, Plant Pathology, College Station, TX, USA. [2]Texas A&M University, Biology, College Station, TX, USA.

We have been developing DNA microarrays to investigate global gene expression and the regulatory networks involved in the control of primary and secondary metabolic pathways associated with trichothecene biosynthesis in *Fusarium sporotrichioides*. Based on our previously generated EST database and results from our early phase DNA microarrays, we have recently constructed an expanded array which contains 300 defined genes. A series of genes which are both positively and negatively coordinately regulated in association with trichothecene biosynthesis have been identified. Detailed information about the expanded arrays and the transcriptional profile data obtained from using them in conjunction with a selected group of toxin mutants will be presented.

488 **Cloning polyketide pigment biosynthetic genes from the chestnut blight fungus *Cryphonectria parasitica* and chemical analyses of pigment mutants.** Alice C. L. Churchill[1], Heather McLane[1], Sagar S. Mungekar[1], Tara Sirvent[2], Stuart Krasnoff[2], Anna Herforth[2], and Donna Gibson[2]. Boyce Thompson Institute[1] and USDA, ARS, Plant, Soil, and Nutrition Laboratory[2], Ithaca, NY.

The orange and yellow pigments of *Cryphonectria parasitica* consist of a family of aromatic polyketides of anthraquinone derivation. Emodin and the other pigments of *C. parasitica* exhibit numerous and diverse biological activities; however, their roles in the biology of the fungus are unknown. We are using insertional mutagenesis (REMI) and PCR amplification of conserved domains of polyketide synthases (PKSs) to clone genes involved in polyketide pigment production in *C. parasitica*. Using REMI, we have isolated thirteen mitotically stable pigment mutants, several of which were isolated as fast- growing sectors from primary transformants. Altered pigment production in many of the REMI mutants generally correlates with significantly reduced asexual sporulation and impaired fertility in sexual crosses. Five of ten pigment mutants failed to produce perithecia in crosses; each of the remaining crosses produced only a few perithecia in comparison with control transformants, which produced numbers of perithecia and levels of pigment comparable to wild type strains. Chemical profiles of several pigment mutants, analyzed *via* HPLC, show significant alterations in amounts and ratios of some of the known pigments; several unknown compounds are being further characterized by HPLC-MS. We have also used PCR amplification, with primers designed to conserved domains of PKSs, to clone putative pigment biosynthetic genes. We have isolated fragments of seven unique genes from *C. parasitica* that show significant sequence similarity to other fungal and bacterial PKS and fatty acid synthase genes. We are conducting complementation analyses of pigment mutants with hybridizing cosmid clones to assess their roles in pigment biosynthesis.

489 **Role of the eukaryotic domain of acetylglutamate kinase in *Neurospora crassa.*** Catherine McKinstry, Richard L. Weiss. UCLA, Chemistry and Biochemistr, Los Angeles, California, USA

Arginine biosynthesis in *Neurospora crassa* is regulated primarily by feedback inhibition of the first two enzymes of the pathway, acetylglutamate synthase (AGS) and acetylglutamate kinase (AGK). Previous genetic studies suggested a coordinate mechanism of inhibition mediated by interaction between AGS and AGK. Mutations in the gene for AGK (*arg-6*) have been identified which affect the activity or feedback sensitivity of AGK and/or AGS. The yeast-two-hybrid system has been used to demonstrate direct interaction between these two enzymes and to define the interaction domain of AGK as a unique C-terminal region found only in the enzyme from eukaryotic organisms. This domain has been termed the eukaryotic domain. *N. crassa arg-6* mutant strains have been transformed with a plasmid encoding AGK lacking the eukaryotic domain. The behavior of the transformants supports an important role for the eukaryotic domain in protein-protein interaction and coordinated feedback inhibition.

490 **Molecular cloning of a gene encoding nucleoside diphosphate kinase and its biochemical properties in *Aspergillus nidulans.*** Nak-Jung Kwon, Hye-Jin Kang[1], Nam-Shik Lee, Seung-Hyen Ka, Kong-Joo Lee[1], and Suhn-Kee Chae. Research Center for Biomedicinal Resources and Division of Life Science, Paichai University, Taejon, Korea, and [1]Center for Cell Signaling Research and College of Pharmacy, Ewha Womans University, Seoul Korea

Nucleoside diphosphate kinase (NDPK) catalyzes the transfer of the terminal phosphate group of a nucleoside triphosphate to a nucleoside diphosphate. Human NDPK-A (nm23-H1) was first isolated as a tumor metastasis

suppressor and coexisted as hetero-hexamers with Nm23-H2, also known as the *c-myc* transcription factor PuF. Autophosphorylation and serine/threonine specific protein phosphotransferase activities were reported for Nm23. However, relevant functions of Nm23 on the various cellular processes are largely unknown. In *Aspergillus nidulans*, we have been isolated a Nm23 homolog gene (*ndkA*) by PCR with a degenerated primer set. *Aspergillus* NDPK consists of 154 amino acids with 65% sequence identity to human Nm23. The NDPK was coded from an ORF of 462bp, interrupted by four introns located on chromosome II. The 1.2Kb transcript was detected in northern analysis and the amount of transcript was decreased during asexual development. Over-expression of the sense and the antisense RNA did not affect growth and differentiation. No evidence on the existence of other isotypes was obtained from non-stringent Southern and western analysis. Biochemical properties of recombinant as well as native NDPK purified using ATP-Sepharose affinity chromatography were examined. *Aspergillus* NDPK was existed as a homo-tetramer (78KDa) judged from gel filtration chromatography. NDPK and autophosphorylation activities were demonstrated and both enzymatic activities were more thermostable than human NDPK. Supported by a grant #1998-001-F00771 from KRF, Korea.

491 **Four clustered and coregulated genes associated with fumonisin biosynthesis in *Fusarium verticillioides*.** Seo, J.-A., Proctor R. H., and Plattner, R.D. USDA ARS NCAUR, Mycotoxin Research, Peoria, Illinois, USA

Fumonisins are mycotoxins that cause several fatal animal diseases, including cancer in rats and mice. These toxins are produced by the maize pathogen *Fusarium verticillioides* (syn. *F. moniliforme*) and can accumulate in maize infected with this fungus. We have identified four *F. verticillioides* genes (*FUM6*, *FUM7*, *FUM8*, and *FUM9*) immediately downstream of *FUM5*, a previously identified polyketide synthase gene that is required for fumonisin biosynthesis. Gene disruption analysis revealed that *FUM6* is required for fumonisin production and Northern blot analysis revealed that expression of the four new genes is correlated with fumonisin production. In contrast, the expression of five genes located upstream of *FUM5* are not correlated with fumonisin production. Nucleotide sequence analysis indicated that the predicted *FUM6* translation product is most similar to cytochrome P450 monooxygenase/reductase fusion proteins, while the predicted products of *FUM7*, *FUM8* and *FUM9* are most similar to type III alcohol dehydrogenases, class-II a-aminotransferases and dioxygenases, respectively. These four groups of enzymes catalyze the same general types of reactions expected to be necessary for fumonisin biosynthesis given the chemical structures of the toxins. Together, these data indicate that *FUM5*, *FUM6*, *FUM7*, *FUM8*, and *FUM9* form part of a fumonisin biosynthetic gene cluster in *F. verticillioides*.

492 **Characterization of a gene cluster with a putative role in toxin production in the ascomycete *Leptosphaeria maculans*.** Alexander Idnurm[1], Janet L. Taylor[2], and Barbara J. Howlett[1]. [1]School of Botany, The University of Melbourne, Parkville, Victoria 3010, Australia. [2]Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, Saskatchewan, S7N 0W9, Canada.

Leptosphaeria maculans is a loculoascomycete that causes blackleg disease of canola (*Brassica napus*) and other *Brassica* species. During a screen of *L. maculans* Expressed Sequence Tags (ESTs), a cDNA was identified with low sequence similarity to *afl*R, the zinc finger regulatory gene for toxins such as aflatoxin and sterigmatocystin production in *Aspergillus* species. Aflatoxins and related compounds have not been reported in *L. maculans*. To investigate the role of this putative regulatory gene in metabolite production in the fungus, a cosmid (34 kb) containing this gene was obtained and the flanking regions of the gene sequenced to seek the presence of other genes that may have a role in biosynthesis of a secondary metabolite. So far, a peptide synthetase gene, similar to those that produce cyclic antibiotics in bacteria, and a transport protein gene have been found. These genes are being mutated in the fungus by targeted gene disruption. Resultant mutants will be screened for the presence/absence of known *L. maculans* metabolites and inoculated onto canola plants to see if these genes play a role in plant disease.

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Unclassified Abstracts

493 **MAP-1 and IAP-1, two novel AAA proteases with catalytic sites on opposite membrane surfaces in the mitochondrial inner membrane of *Neurospora crassa*.** Holger Prokisch, Carola Klanner and Thomas Langer. Adolf-Butenandt-Institut für Physiologische Chemie, Universität München, Goethestrasse 33, 80336 München, Germany

Eukaryotic AAA proteases form a conserved family of membrane-embedded ATP-dependent proteases but have been analyzed functionally only in the yeast *Saccharomyces cerevisiae*. Here we have identified two novel members of this protein family in the filamentous fungus *Neurospora crassa* which were termed MAP-1 and IAP-1. Both proteins are localized to the inner membrane of mitochondria. They are part of two similar-sized high molecular mass complexes, but expose their catalytic sites to opposite membrane surfaces, namely the intermembrane and the matrix space. Disruption of iap-1 by repeat-induced point mutation caused a slow growth phenotype at high temperature and stabilization of a misfolded inner membrane protein against degradation. IAP-1 could partially substitute for functions of its yeast homologue Yme1 demonstrating functional conservation. Deficiencies of Dyme1 yeast cells in mitochondrial morphology as well as in cell growth at low temperature were suppressed upon expression of IAP-1, whereas respiratory growth at 37°C was not restored. Our results identify two components of the quality control system of the mitochondrial inner membrane in *N. crassa* and suggest that AAA proteases with catalytic sites exposed to opposite membrane surfaces are present in mitochondria of all eukaryotic cells.

494 **Developing *Mycosphaerella graminicola* as a model fungal plant pathogen for analysing gene function.** Wendy Skinner, John Keon, John Hargreaves. IACR-Long Ashton Research Station

Mycosphaerella graminicola (anamorph *Septoria tritici*), the causal agent of septoria leaf blotch disease on wheat, is an economically important pathogen throughout the world. Little is known about the mechanisms by which this fungus invades and destroys its host. Understanding the genetic determinants that influence the pathogenic habit would greatly expand our knowledge of this disease and may provide clues for the development of novel and environmentally benign control measures.
The genetic tools required for unravelling the basis of pathogenicity in a filamentous fungus include large-scale mutagenesis systems, rapid and reliable infection assays conducive to high through-put screening, a sexual crossing cycle to establish the segregation of the mutation during
recombination, and an efficient transformation system for subsequent genetic manipulation (Sweigard *et al*., 1998). Here we report on our progress towards developing protocols for *M. graminicola* for each of these requirements.
Reference: Sweigard, J. A., Carroll, A. M., Farrall, L., Chumley, F. G. & Valent, B.
(1998). *Magnaporthe grisea* pathogenicity genes obtained through insertional mutagenesis. Molecular Plant-Microbe Interactions 11:404-412.

495 **Chitin synthases of *Coccidioides immitis*, the valley fever fungus.** Alejandra M. Mandel[1], Kris I. Orsborn[2], John N. Galgiani[2] and Marc Orbach[1]. [1]University of Arizona, Plant Pathology, Tucson, AZ USA. [2]University of Arizona, Valley Fever Center, Tucson, AZ USA

Coccidioides immitis is the causal agent of Valley Fever, a disease of humans and other mammals. The infection cycle is initiated when arthroconidia (single cells, approximately 5 microns in size, the product of filamentous saprobic soil growth) are inhaled into the lung and the fungus begins parasitic growth as spherules (multicellular structures, 50 microns or larger in diameter). This morphologic switch must involve the formation of new cell wall. NikkomycinZ, an antifungal that variably inhibits some chitin synthases, is exceptionally therapeutic in experimental murine coccidioidal infections. This suggests that chitin in the spherule cell wall is critical to pathogenicity. These observations have led us to analyze the role of different chitin synthases in spherule morphogenesis. We have isolated and sequenced completely four CHS genes from *C. immitis*, corresponding to

classes I to IV (according to Robbins et al, 1992). These CHSes share similarity with other fungi. In some cases, intron number and positions are shared with other fungi and in other cases they are not. We are disrupting each CHS gene using Agrobacterium-mediated transformation to determine their roles in growth. Each mutant strain will be screened for differences from normal growth in both the saprobic and parasitic phases, with particular interest in defects in their ability to properly form spherules. If a mutant is found to be less sensitive to nikkomycin Z than the wild-type, this may identify the drug's therapeutic target. We are also analyzing the transcription patterns of these genes during the infection cycle of *C. immitis*. By defining which CHS genes are responsible for spherule morphogenesis, this research should provide a basis for future studies of control.

496 **Characterization of Constiutively Active RAS and CAAX Box Deletion Mutants of *C.trifolli.*** S.Memmott, Y.Ha and M.Dickman, Department of Plant Pathology, University of Nebraska-Lincoln, NE 68503-0722

Colletotrichum trifolii is the causative organism of alfalfa anthracnose. In order to characterize the signaling pathways involved in the interaction between this pathogen and its host we previously cloned the small prototypical G-protein, RAS of *C.trifolii*. Transformants expressing constitutively active (oncogenic) forms of RAS express differential phenotypes, when compared to wild type, which were dependent on the growth media. In nutrient rich media, (eg yeast extract, peptone) the phenotype of the transformants was indistinguishable form wild type. However, under conditions of nutrient starvation, the transfomants exhibited a loss of polarity, distended hyphae, failure to sporulate and produce appressoria. Studies have been conducted to identify the component(s) responsible for reversing the aberrant phenotype observed in minimal media. We have found that proline is responsible for the reversal of the constitutively active RAS phenotype. To understand the mechanism underlying this response, proline analogs have been utilized to see if they gave the same phenotypic reversal. Amino acid analyses of cytosolic extracts are being performed to determine differences in amino acid synthesis and or degradation between strains containing the wild type or constitutively active forms of RAS.

Preliminary studies using the farnesyl-protein transferase inhibitor fusidienol A suggested that the localization of RAS is essential to germination and growth. In the case of wild type and constitutively active RAS strains, germination rates were severely curtailed and germlings were identified that exhibited a distended morphology and stopped growing after 48 hours. To better determine that mislocalisation of RAS is responsible for this phenotype, a RAS clone containing a CAAX box deletion under the control of an inducible promoter was made and transformed into wild type *C.trifolli.* Under inducing conditions transformants were similar in phenotype to those treated with fusidienol A supporting the idea that proper localization of RAS is necessary for normal growth.

497 **Colletotrichum trifolii TB3 kinase, a COT-1 homolog, is light-inducible and nuclear-localized.** Changbin Chen and Martin B. Dickman; Department of Plant Pathology, University of Nebraska-Lincoln, NE68583-0722

Colletotrichum trifolii is a fungal pathogen responsible for anthracnose disease of alfalfa. A serine/threonine protein kinase gene TB3, which is a functional homolog of *Neurospora crassa* COT-1, has been previously isolated in our lab and appears to be associated with hyphal elongation and branching in the same manner as COT-1. Since COT-1 is light regulated, we evaluated the effect of light on hyphal growth and TB3 expression. Results indicated that similar to COT-1, hyphal branching frequency is increased and TB3 expression is rapidly induced following illumination. It has been shown that COT-1 is expressed in the cytoplasm, membrane and nucleus. Western analysis using TB3 antibodies showed expression in both cytoplasm and nucleus, but not in membrane fractions. Moreover, indirect immunofluorescence indicated that TB3 is abundantly found in the nucleus. In order to detect the subcellular distribution of TB3 protein, we inserted a TB3::GFP fusion construct into *Colletotrichum trifolii*. Results indicated that the cellular distribution of TB3 changes during developmental transitions. Consistent with our previous observations, TB3 was localized in both the cytoplasm and nucleus but was preferentially abundant in the nucleus during hyphal growth. The amino terminus of TB3 contains several polyglutamine repeats which are absent in COT-1; such tracts are associated with protein-protein interactions, particularly with respect to transcriptional activation. We postulate that TB3 may be positioned in a signaling cascade resulting in proper hyphal growth and development by functioning as a transcription factor.

498**A plant cutin activate protein kinase from *C. trifolii* is required in appressorium development.** Youngsil Ha, C. Huang, Z. Yahg and Martin B. Dickman. Department of Plant Pathology, University of Nebraska-Lincoln, NE

Colletotrichum trifolii causes anthracnose of alfalfa. Successful infection is predicated on appressorium development. Pharmacological data indicated that a protein kinase C like gene product was necessary for appressrium formation. Using degenerate primers and PCR, we have identified a PKC like gene encoding 72kd protein in *Colletotrichum trifolii*. The deduced amino acid sequence of this protein revealed that it has a high degree of similarity to protein kinase C-like proteins of other fungi but only in the catalytic domain. However, we were unable to induce enzyme activity in the presence of PKC activators, diacylglycerol or phorbol esters. Interestingly, we found that the gene expression could be rapidly incuced by plant cutin and its fatty acid monomeric constituents. Structurally similar fatty acids, which are not found in cutin, were uanable to induce the expression of this gene. Over-expression of this Lipid Activated Protein Kinase (LAPK) resulted in abberrant formation of multiple appressoria. Gene disurption of LAPK resulted in the inability of *C. trifolii* to form appressorium and such transformants were incapable of infecting alfalfa. Thus the fungus uses host plant cues for induction of gene expression required for pathogenic development.

499**Transcriptional control of the cellulase genes in *Trichoderma* Sp. strains FB 1231.** Rojas T.[1], Rosales RA.[1,2], Pérez J.[1], Villaverde M.[1], Ortiz E.[1,3], and Nuñez R[3]. [1]Faculty of Biology, La Havana University, 25 Esq. I. Havana. Cuba. [2]Faculty of Sciences, State University, Iguá S/N. Montevideo Uruguay. [3]Institute of Oceanology. 1ra. No. 18408. Playa. Havana. Cuba

The expression of the cellulase transcripts of Trichoderma sp. strains FB 1231 is controlled by the nature of the energy carbon sources used in the culture medium. Cellulose and the soluble disaccharide sophorose, but not glycerol or glucose, act as inducers. Evidence is presented suggesting that a low constitutive extracellular cellulolytic system catalyses the formation of a soluble inducer from cellulose, and this inducer triggers the expression of the cellulose transcripts. This basal and cellulose-induced expression of the cellobiohydrolase I mRNAs (cbh1), the major member of the cellulose system, is transcriptional controlled by two independent cis-acting DNA regions. In addition, expression of the cbh1 transcript is influenced by the physiological state of the mitochondria and this sensitivity is controlled through the 5´-flanking DNA sequence of this gene.

500 **The *Ustilago maydis* sequencing project**

Jörg Kämper[1], Gerhard Weinzierl[1], Andreas Brachmann[1], Michael Feldbrügge[1], Christoph Basse[1], Gero Steinberg[1], Regine Kahmann[1], Gabi Friedrich[2], Verena Vollenbroich[2], Edda Koopmann[2], Isolde Häuser-Hahn[2], Dirk Nennstiel[2], Kai Sievert[2], Rüdiger Suelmann[2], Martin Vaupel[2], Christian Aichinger[3], Ronald Ebbert[3], Birgitta Leuthner[3], Birgit Jaitner[3], Volkhart Li[3], Peter Schreier[3], Thomas Schlüter[4], Dagmar Schütte[4], Harald Kranz[4], Jürgen Henrich[4], Günter Kurapkat[4], Monika Arenz[4], Hartmut Voss[4]. [1] Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str., 35043 Marburg, Germany. [2] Central Research, Bayer AG, 51368 Leverkusen, Germany. [3]Agricultural Center Monheim, Bayer AG , 51368 Leverkusen, Germany [4]Lion Bioscience AG, Im Neuenheimer Feld 515-517, 69120 Heidelberg, Germany

The phytopathogenic fungus *Ustilago maydis* is the causal agent of smut disease on corn. We have sequenced the genome of *U. maydis* strain 521 which was estimated to comprise 20.5 Mb to foster a molecular understanding of pathogenicity and fungal development. In addition, the sequence will allow the development of novel strategies for the identification of anti-fungal compounds.

Using high throughput hybridization technologies, 258 BAC clones were assembled in 28 contigs representative for the 23 *U. maydis* chromosomes. Prior to sequencing an ordered set of overlapping plasmid subclones was generated for each BAC. A total of 17.425.931 base pairs were sequenced, covering approximately 85.9% of the genome. The systematic combination of this genome analysis with the sequencing of 2626 unique cDNA clones allowed to access more than 90% of all *U. maydis* genes. For the identification of potential protein coding regions an automated bioinformatic analysis was used. From a total of 6700 regions with coding capacities, more than 60 % of the open reading frames show significant similarities to entries in protein databases. At least 5 distinct groups of retrotransposons were identified, while no DNA transposons were found. In addition, several duplicated sequences up to 50 kb were identified, a finding that can possibly be linked to the genome variability observed between different *U. maydis* strains.

501 **Activity of transposable element Restless in *Neurospora crassa*** Frank Windhofer[1], David E.A. Catcheside[2], Frank Kempken[1]. [1]Ruhr-Universitat Bochum, Allg & Molekulare Botanik, Bochum, Germany. [2]Flinders University, School of Biol Science, Adelaide Australia

In the past years several fungal transposable elements have been identified (Kempken and K ck, 1998, Bioassays 20:652-659). We have isolated and characterized *Restless*, a new type of fungal class II transposons from *Tolypocladium inflatum* (synonym: *Beauveria nivea*) which so far has not been found in any other fungus (Kempken and K ck, 1996, Mol Cell Biol 16:6563-6572). The predicted amino acid sequence deduced from an open reading frame encoded by *Restless* shows significant homology to transposases of the *hAT* transposon family, e.g. the maize *Activator* element. We were able to proof the usefulness of *Restless* as a molecular tool to tag and identify a new gene in *T.inflatum* (Kempken and K ck, 2000, Mol Gen Genet 263:302-308). Here we present new data regarding the activity of the transposon *Restless* in a foreign host, i.e. *Neurospora crassa*. We show that multiple copies of the element introduced by transformation are subject to methylation, whereas single copies integrated at the *his-3* locus are not subject of methylation (Windhofer et al. 2000, Curr Genet 37:194-199). Single copy *Restless* transformants were selected for transposition of *Restless*. Numerous excision events have already been identified and characterized. Currently the ability of *Restless* to reintegrate into new genomic locations is under investigation.

502 **Functional Analysis of a Cdc42 Ortholog from *Magnaporthe grisea*.** Zhiying Zhao[1], Zonghua Wang[2], Peter Albersheim[1], Alan G Darvill[1], Zhenbiao Yang[3], Shengcheng Wu[1]. [1]University of Georgia, Complex Carbohydrate Research Center, Athens, Georgia, USA. [2]Fujian Agricultural Univ, Plant Protection, Fuzhou Fujian China. [3]University of California, Botany and Plant Sciences, Riverside California USA

Following contact with a plant surface, spores of some phytopathogenic fungi produce by polar growth a germ tube, which differentiates into a highly pressurized dome-shaped appressorium. The appressorium then develops a penetration peg, which pierces the plant surface and starts the infective growth phase. Understanding the mechanism by which such invasive polarized structures are formed may lead to the development of novel methods for fungal disease control. We have cloned from Magnaporthe grisea, a notorious fungal pathogen of rice, mgCdc42, an ortholog of Cdc42 GTPase that regulates polar growth, bud emergence and pseudohyphal growth in yeast. mgCdc42 complements a lethal cdc42 temperature-sensitive mutant of Saccharomyces cerevisiae. However, in contrast to the case in S. cerevisiae, mgcdc42 knockout mutants of M. grisea are viable and do not exhibit obvious deficiency in germination and hyphal polar growth. However, these mgcdc42 mutants are only lightly pigmented on oatmeal agar, and produce gherkin-shaped conidia rather than the bulb-shaped wild-type ones. Under glass coverslip, wild-type conidia germinate and differentiate appressoria at almost 100% after 6 hours of incubation, whereas mgcdc42 mutants do not produce any. Based on these preliminary results, mgCdc42 appears to play a role in M. grisea different from that in yeast. Further analyses of the mgcdc42 mutants will be presented and discussed at the conference. (This work was supported in part by a grant from Natural Science Foundation of Fujian, China, and by U. S. Department of Energy grant DE-FG05- 93ER20114 and the DOE-funded (DE-FG05-93ER20097) Center for Plant and Microbial Complex Carbohydrates.)

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