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22nd Fungal Genetics Conference at Asilomar

Abstract

Abstracts from the 2003 Fungal Genetics Conference at Asilomar

22nd Fungal Genetics Conference at Asilomar

Plenary Session Abstracts

Microtubules in polar growth of *Ustilago maydis*

Gero Steinberg

Polar growth of yeasts and filamentous fungi depends on cytoskeleton-based transport of vesicles and protein complexes towards the expanding cell pole. While solid evidence exists for a central function of F-actin in fungal growth, the importance of the tubulin cytoskeleton is by far less understood. In vivo observation of microtubules in growing cells of *Ustilago maydis* revealed that the dynamic ends ("plus"-ends) of these tubulin polymers are growing towards the cell poles, while cytoplasmic microtubule organizing centers focus the microtubule minus-ends at the neck region. This microtubule orientation suggests that minus-end directed dynein motors support transport towards the neck, whereas plus-end directed kinesin motors might be required for transport towards the poles of the cell. This concept is supported by the recent identification of the transport machinery for microtubule-based traffic of early endosomes that depends on both a Kif1A-like kinesin and cytoplasmic dynein. Interestingly, a balance of the activity of these motors determines the cell cycle-dependent formation of endosome clusters at both cell poles, where the endosomes apparently support polar growth, bipolar budding and septation. However, it emerges that, in addition to their function in membrane transport, both motors influence the organization of their transport "tracks" by modifying dynamic parameters of microtubules in *U. maydis*. Understanding this dual function of motors will be a fascinating future challenge, and might be key to the understanding of the role of microtubules in polar fungal growth.

Genetics analysis of the regulation of cytoplasmic dynein in Neurospora.

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Cytoplasmic dynein is the most complex of the cytoplasmic microtubule-associated motor proteins, and it has been shown to be required for retrograde vesicle transport and the movement and positioning of nuclei in fungi. Analysis of cytoplasmic dynein from mammals reveals that at least 16 different gene products are required for formation of the dynein motor, the dynein activator complex dynactin, and the LIS1 complex, required for dynein function. Examination of the recently completed genome sequence of *Neurospora crassa* reveals a high degree of conservation for nearly all these gene products. We have developed a simple genetic system for the isolation of mutants defective in cytoplasmic dynein function. We have shown previously that most of the genes defined through this screen encode subunits of cytoplasmic dynein or dynactin. We have now cloned all but one of the genes defined by our screen. Interestingly, we have not identified mutations in four genes encoding known dynein/dynactin subunits, although genomic sequence data suggests that apparent orthologs are present in *N. crassa*. In addition, we have also defined two genes that have not previously been implicated as required for dynein/dynactin function.

Septins in *Aspergillus nidulans*.

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Septins form scaffolds that organize division sites and areas of new growth in fungi and animals. Many proteins critical for cytokinesis and cell cycle regulation depend upon septins for proper localization. Five septins (AspA-AspE) have been identified in *A. nidulans*. AspB localizes to forming septa and conidiophore layers. It also localizes to emerging branches where it may play a role in cell cycle regulation of subapical compartments. Deletion of AspB is lethal and deletion of other septins results in branched conidiophores.

Nuclear migration in *Aspergillus nidulans*: Motors, microtubules and more

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Nuclei migrate through growing hyphae of *A. nidulans* with a similar speed as hyphae elongate. However, nuclear behavior is much more complex than just a simple tipward travel. They oscillate on their way around a certain position, move backwards for short time periods, enter newly formed branches, devide while moving and populate the reproductive structures such as asexual and sexual spores. The movement requires the microtubule-dependent motor dynein and an intact microtubule cytoskeleton. We asked whether kinesins may play a role in the migration process and isolated three novel genes. The corresponding proteins displayed similarity to conventional kinesin and *S. cerevisiae* Kip2 (KipA) and Kip3 (KipB). Deletion analyses revealed that only conventional kinesin had an effect on nuclear migration. KipA-mutation affected polarized growth, likely through the positioning of the Spitzenkörper and KipB appeared to be involved in mitosis and meiosis. Likewise, the latter protein localised to cytoplasmic and to mitotic microtubules.

In addition to motor proteins and the microtubule tracks, nuclear positioning in *A. nidulans* requires two proteins, ApsA and ApsB. *In vivo* localisation studies revealed that ApsA forms a protein gradient along the cortex. This gradient depends on the actin cytoskeleton to be maintained. In contrast, ApsB localised to the nucleus and appeared to be associated to the spindle pole body. Simultaneous observation of nuclear movement, microtubule dynamics and ApsB behavior suggested that ApsB might be invovled in the movement of nuclei along microtubule filaments. We observed migration of the nucleus along one microtubule filament towards the plus and the minus end of one microtubule, suggesting the involvement of motors with different polarity. We believe that this movement represents one possibility how nuclei can be translocated within a hyphal cell.

Fungal mitosis, closed or open?

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The nuclear envelope is broken down at mitosis in higher eukaryotes leading to an "open" mitosis but in fungi the mitotic apparatus is enclosed within a nuclear envelope and so remains "closed". Recent work on the NIMA kinase of *Aspergillus nidulans* suggests that the closed mitosis of fungi may be more open than previously recognized.

We have determined the location of NIMA in real time through the cell cycle. At G2 it is excluded from the nucleus and at early mitosis it locates transiently to the nuclear pore complex (NPC) before locating to the nucleus. The C-terminal regulatory domain of NIMA is sufficient to target GFP to the NPC and this domain acts in a dominant negative fashion, delaying entry into mitosis. The data support a mitotic role for NIMA at the NPC.

We have previously isolated *sonA* as a NPC protein that could specifically suppress *nimA1*. A second extragenic suppressor of *nimA1* has now been cloned (*sonB*) and identified as a NPC protein with homology to human Nup98/Nup96 proteins. Not only do mutations of *sonA* and *sonB* suppress the *nimA1* mutation, these two NPC proteins physically interact as revealed by co-immunoprecipitation experiments. Moreover, the interaction between SONA and SONB is reduced by the *sonB1* mutation which is within a domain of SONB responsible for binding to SONA. Thus, both genetic and biochemical data indicate that NIMA and two interacting NPC proteins play a role in mitotic regulation.

The dynamic localization of SONA and SONB further implicates these proteins in mitotic regulation, as both are precipitously lost from the NPC at mitotic onset and then locate back to the NPC at mitotic exit. These studies reveal for the first time that the protein makeup of the NPC radically changes during a closed fungal mitosis. We propose that these changes open the NPC to allow protein diffusion either in or out of nuclei during mitosis. Proteins which have an affinity for nuclear components concentrate within nuclei, for example tubulin and NIMA. Other nuclear proteins, with no affinity for nuclear components, escape from nuclei during mitosis into the cytoplasm. As daughter nuclei are produced, and SONA and SONB relocate to the NPC, normal nuclear transport is reestablished. This then plays a role in relocating proteins by active transport. Therefore, perhaps the "closed" mitosis of fungi is more open then previously realized.

Distinct endophyte genome evolution associated with differing degrees of antagonism or mutualism

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Epichloë (anamorph = *Neotyphodium*) species are systemic and constitutive fungal symbionts (endophytes) of cool-season grasses (subfamily Poöideae). This single genus includes pathogenic and mutualistic symbionts. The more mutualistic endophytes transmit by infection of floral meristems and, ultimately, embryos (vertical transmission); the more pathogenic species transmit horizontally via ascomata that surround and abort host inflorescences (choke disease). In mixed transmission, only some inflorescences are choked, whereas others produce endophyte-bearing seeds. Vertical- and mixed-transmission *Epichloë/Neotyphodium* species are typically host specific, whereas more pathogenic species can have broader host ranges. Our recent phylogenetic analyses of endophyte and host species indicate a tendency toward co-phylogeny. However, our results also suggest that major host shifts often lead to loss of sexual expression, resulting in symbiota in which the endophyte (much like an organelle) is disseminated only via its host maternal lineage. Should a second *Epichloë* species superinfect such a symbiotum, the resident endophyte and newcomer may hybridize. Two thirds of the asexual endophytes characterized to date are interspecific hybrids, and repeated hybridizations are evident in some endophyte pedigrees. This, and the observation that hybrids tend to dominate symbiont populations in many host species, strongly suggest a fitness advantage conferred by hybridization. Possible bases for this advantage are (1) as a counter to Muller's ratchet, and (2) pyramiding of genes that enhance host and, therefore, endophyte fitness. Examples of such fitness-enhancing genes include those that direct the biosynthesis of anti-herbivore alkaloids, and genes yet to be identified for drought tolerance, nematode resistance, and improved nutrient acquisition.

Of fungi and plants: specific developmental and metabolic processes required for their interactions

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Fungi have developed various strategies to attack plants. In most cases, however, early stage of the infection is marked by the differentiation of specialized structures, such as appressoria, that mediate the penetration of the fungus into host tissues. This process requires specific developmental programs and metabolic pathways that have been highlighted in the rice blast fungus *Magnaporthe grisea*. Analysis of a non-pathogenic mutant, defective for a B-zip transcription factor that is specifically expressed during infection, suggests the existence of infection specific regulatory networks. Infection-specific regulatory networks, as shown for *ACE1*, also control avirulence gene expression. Here, at least two different signals are involved in the control of *ACE1* expression. The tetraspanin-encoding gene *PLS1*, that is essential for penetration, also displays an appressorium-specific expression. Control of *PLS1* expression occurs at the post-transcriptional level and involves its 5'UTR sequence. Genome wide transcriptome analysis of mutants defective for host penetration that are involved in signaling pathways such as *PLS1*, or defective for infection-specific expression, should reveal the networks involved in the infection process. Although most appressorium signaling/regulatory pathways were identified in *M. grisea*, recent experiments suggest that these pathways are involved in the infection process of other fungal species.

Signal transduction in *Ustilago maydis*: for mating and more

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Ustilago maydis is a dimorphic fungus that switches from a yeast-like haploid stage to a filamentous dikaryon after mating of sexually compatible strains. In nature it is the dikaryon that is able to differentiate infection structures and cause disease.

To understand the complex signaling processes involved in cell fusion and pathogenicity molecular tools for functional genome analysis were applied. These include REMI, the use of regulated promoters in differential screens as well as the exploitation of DNA micro arrays based on the annotation of the *U. maydis* genomic sequence. I will discuss these strategies and highlight their impact for the identification of pathogenicity determinants in this system. Special emphasis will be given to signaling molecules derived from either the fungus or the plant.

New strategies for *C. albicans* virulence gene discovery

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Candida albicans is the most frequently encountered fungal pathogen of humans. Although it is a benign inhabitant of mucosal surfaces in most individuals, it is a significant cause of infection when host or environmental factors are permissive. Much effort has focused on the definition of genes that govern *C. albicans* survival in the host and pathogenicity. Most known *C. albicans* survival and pathogenicity genes have been identified through their expression patterns, through properties of their gene products, or through phenotypes that arise from overexpression or heterologous expression in *Saccharomyces cerevisiae*. Ultimately, gene function is deduced from disruption mutations that cause loss-of-function defects. However, it has been largely impossible to identify genes through an initial screen of gene disruption mutants because *C. albicans* is an asexual diploid, so that creation of homozygous mutants requires involved genetic manipulations.

We report the results of an insertional mutagenesis of *C. albicans* for virulence gene discovery. We have described a gene disruption cassette, *UAU1*, that permits selection for homozygous mutants after insertion in a single allele. *UAU1* was incorporated into a bacterial *Tn7* transposon to permit in vitro mutagenesis of a genomic library. Insertions in *C. albicans* ORF sequences were then transformed into *C. albicans* to create a set of *orf::Tn7/orf::Tn7* insertion mutants. We have thus far disrupted 217 ORFs, and have evidence that another 36 ORFs may be essential for viability. This collection is far from comprehensive – there are ~7110 ORFs in the *C. albicans* "haploid complement" – but screening of these mutants has permitted identification of new genes required for host cell damage, pH-dependent filamentation, azole drug resistance, biofilm formation, and other unique *C. albicans* biological properties.

Melanin biosynthesis and virulence of the human-pathogenic fungus *Aspergillus fumigatus*

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Aspergillus fumigatus is an important opportunistic human-pathogenic fungus that causes invasive aspergillosis (IPA) in immunocompromised patients. Since conidia are the infectious agent in IPA, we focused on the elucidation of conidial factors contributing to pathogenicity. Previously, we isolated a mutant of *A. fumigatus* which lacked the ability to form the grey-green pigment characteristic of wild-type conidia. Conidia of this mutant are white. Cloning of the gene defective in the mutant led to the identification of a gene designated *pksP* (=*alb1*) for polyketide synthase involved in pigment biosynthesis. Conidia of a *pksP* mutant strain showed reduced virulence in a mouse infection model and were 10-20-fold more sensitive against reactive oxygen species (ROS) compared with wild-type conidia. Wild-type conidia were able to scavenge ROS, presumably thereby detoxifying ROS. The *pksP* gene was found to be part of a cluster which is involved in the biosynthesis of 1,8-dihydroxynaphthalene (DHN)-melanin present in conidia. The analysis of an P*pksP-egfp* gene fusion in *A. fumigatus* showed that the *pksP-egfp* gene fusion was expressed *in vivo* in outgrowing hyphae isolated from the lungs of infected immunocompromised mice. Furthermore, our data suggest that the presence of a functional *pksP* gene in *A. fumigatus* conidia is associated with an inhibition of phagolysosome fusion in human monocyte derived macrophages (MDM). In summary, these findings provide a conceptual frame to understand the virulence of *A. fumigatus*.

Fatal Attraction: Heterokaryon incompatibility in *Neurospora*.

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Filamentous fungi grow by tip extension, branching and hyphal fusion (anastomosis) to form a hyphal network that makes up a fungal individual. In addition to "self" hyphal fusion, filamentous fungi are capable of undergoing hyphal fusion between individuals to make heterokaryons. Recognition of nonself in such heterokaryons is mediated by genetic differences at het (for heterokaryon incompatibility) loci. Heterokaryons or partial diploids that contain alternative *het* alleles show severe growth inhibition, suppression of conidiation and hyphal compartmentation and death, a phenomenon reminiscent of programmed cell death in other organisms. In *Neurospora crassa*, nonself recognition mediated by the *het-c* locus occurs by the formation of a HET-C heterocomplex, which is associated with the plasma membrane of dead hyphal compartments. Targeting of HET-C to the plasma membrane is not essential for HET-C heterocomplex formation or aggregation. Genetic analysis to identify mutations that suppress heterokaryon incompatibility revealed that a MAPK signal transduction pathway and a putative transcription factor are required for heterokaryon incompatibility. Some of the suppressor mutations also affect heterokaryon incompatibility mediated by other *het* loci. These data suggest that common cellular machinery is involved in mediating heterokaryon incompatibility by allelic differences at a number of *het* loci; hypotheses for how this death pathway may be activated can now be tested.

Establishment and maturation of hyphal growth in the filamentous ascomycete *Ashbya gossypii*

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A. gossypii was originally isolated as cotton pathogen. It gained attention as a model organism for the analysis of filamentous growth due to a few unique features. Linear transforming DNA is exclusively integrated by homologous recombination allowing efficient PCR-based gene targeting. Circular transforming DNA carrying a replication origin of the yeast *S. cerevisiae* can autonomously replicate in *A. gossypii* allowing homologous and heterologous complementation studies. The completely sequenced genome consists of only 9MB coding for 4720 proteins and each nucleus carries only one copy of this genome. 95% of the *A. gossypii* genes are homologues of *S. cerevisiae* genes, very often with conserved gene order (synteny).

Despite the apparent evolutionary relation to *S. cerevisiae* the hyphal growth of *A. gossypii* is typical for filamentous fungi. Spores develop into germlings which grow by sustained tip extensions, lateral branching and, at a later stage, by tip branching. The growth speed of hyphal tips can increase from 0.01mm /hour (germling) up to 0,25mm /hour in mature mycelium.

We identified in the *Ashbya* genome homologues to all *S. cerevisiae* genes known to be important for polar growth control. We also found an additional copy of a formin gene, a *RHO* gene, and a *GEF* gene. Several of these genes were deleted and/or fused to the GFP coding sequence. Analyses of growth dynamics of theses strains using video microscopy allowed important conclusions about polar growth control in *A.gossypii*. Key results of these studies will be presented.

PKC regulates the stability of WC-1 in response to light

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Previous pharmachological studies have shown that *Neurospora* Protein Kinase C (PKC) is involved in the regulation of the light responsive genes. We have studied the function of PKC in the light response by investigating its biochemical and functional interaction with the blue light photoreceptor WC-1. We demonstrate that WC-1 and PKC interact in a light regulated manner *in vivo*, and that immunopurified PKC phosphorylates WC-1 zinc-finger region *in vitro*. In addition, we show that the mutated PKCs induce changes in WC-1 protein levels. A dominant negative PKC induces a dramatic increase in WC-1 protein levels, which is the result of slower WC-1 degradation rate. Consistently, in the presence of a constitutively active PKC we see decreased levels of WC-1. We show that the altered photoreceptor levels induced by PKC cause an altered light induced transcription of the *al-2* mRNA. We see opposite effects in the presence of the dominant negative PKC. In addition we tested PKC effects on FRQ protein levels and

degradation rate, and we see that that PKC induces a change in FRQ levels, affecting the robustness of the circadian rhythm. Together our data indicate that PKC is a novel component of the *Neurospora* light signal transduction pathway physically interacting with and phosphorylating WC-1.

Listening to Silenced Genes

Robert L. Metzenberg and Patrick K.-T. Shiu

Filamentous fungi occupy a unique position in the hierarchy of cellular organization. Their nuclei are self-contained like those of other microorganisms; they do not ordinarily undergo irreversible differentiation of function. However, their undivided cytoplasm is shared by thousands to many millions of nuclei, and from this point of view, fungi are thoroughly macroscopic, ranging in size from centimeters to kilometers. This suits them well in their niche, but it also leaves them as extraordinary targets of opportunity for the spread of viruses and retrotransposons.

It is not surprising, then, that fungi, here exemplified by *Neurospora crassa*, have evolved an array of mechanisms for combatting the proliferation of these renegade elements. Two of these, Repeat-Induced Point-mutation (RIP) and quelling, have been elegantly explored by Selker and his coworkers and by Macino and Cogoni and their group. Despite the extreme dissimilarity of these processes, they have one feature in common: sequences present in two or more copies in a cell which should contain only one copy are recognized as aberrant, and their effects are nullified. RIP occurs in a window of time in which pre-karyogamic nuclei reside in ascogenous hyphae. It introduces numerous GC to AT transitions into any DNA present in more than one copy in a nucleus, which in general, inactivates the gene or transposon irreversibly. Quelling, by contrast, operates during the vegetative phase of life. It acts against aberrant RNA molecules by fragmenting them, but not directly against the genes that gave rise to them. Both RIP and quelling presumably judge a sequence as illegitimate by the fact that it must have transposed at least once.

We have described another mechanism that could silence potentially damaging genetic elements. This process, Meiotic Silencing by Unpaired DNA (MSUD), operates primarily, but perhaps not exclusively, in the brief period following karyogamy. During pachytene, legitimate DNA sequences of a chromatid will be diploid, each copy being paired with those of a homolog. Any sequence that does not have a pairing partner at a corresponding position of its homolog is taken as "aberrant" and is silenced by a mechanism that involves double-stranded RNA. Furthermore, any sequence homologous to the unpaired DNA is silenced by MSUD, even if it is itself properly paired with a homolog. Thus, unlike RIP and quelling, MSUD has the properties necessary to silence a virgin transposon, but cannot act against a quiescent transposon that has established itself in canonical positions of both parents, as can RIP and quelling.

Investigating the genetics of appressorium-mediated plant infection by *Magnaporthe grisea*. Nicholas J. Talbot, Sara L. Tucker, Martin Egan, Karen Tasker, Martin J. Gilbert, and Darren M. Soanes. School of Biological Sciences, University of Exeter, Exeter, EX4 4QG, UK

Magnaporthe grisea infects its host by elaborating a specialised infection structure known as an appressorium. This cell forms in response to the hard, hydrophobic rice leaf surface and brings about infection by generation of hydrostatic pressure. *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 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 plant leaf tissue. We are investigating mechanisms required for appressoria to form and the genetic control of appressorium formation via the PMK1 MAP kinase pathway. Subsequent to appressorium maturation, we are studying the origin of appressorial glycerol, its biosynthetic pathway during appressorium formation and genetic regulation of turgor generation. In particular the role of trehalose metabolism, lipid metabolism and glycogen metabolism during turgor generation are being investigated. The availability of substantial EST sets and the recent first draft of the M. grisea genome sequence is allowing us to adopt a more holistic approach to investigation of appressorium differentiation and function. We are therefore developing bioinformatic tools to compare *M. grisea* and related pathogenic fungi with free-living saprotrophic relatives.

Neurospora biology, from the genome up

Bruce Birren and the sequencing group at the Whitehead Inst./MIT Center for Genome Research, and the Neurospora Community Analysis Project.

A collaborative effort between the Genome Sequencing and Analysis Program at the Whitehead Genome Center and members of the Neurospora research community has produced and analyzed a high-quality draft sequence of the *Neurospora crassa* genome. The complexity of the filamentous fungi relative to the previously sequenced yeasts is highlighted by the number and kinds of genes we find. Neurospora contains ~10,000 protein-coding genes. This is more than twice the number found in the fission yeast *S. pombe* and only about 25% fewer than in the fruit fly *D. melanogaster*. Despite the decades of genetic analysis of this organism, the predicted gene set suggests significant biological processes remain to be explored in Neurospora. These include the potential for red light photobiology, secondary metabolism, and important differences in Ca2+ signaling as compared to plants and animals. The genome sequence has also permitted a global analysis of the process of Repeat Induced Point Mutation (RIP). Sequence analysis indicates that RIP has greatly slowed the creation of new genes through duplication and as a consequence Neurospora contains an unusually low proportion of closely related genes.

Neurospora represents the first of numerous fungal genomes that are being sequenced at the Whitehead Genome Center. The Fungal Genome Initiative will sequence a comprehensive collection of species spanning the entire fungal kingdom. These data will allow all fungal researchers to apply the power of comparative genomics to further our understanding of the remarkable diversity of these organisms.

Comparative genomics of plant pathogenic fungi

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Access to complete genomes of saprobic and pathogenic euascomycetes has allowed us to compare inventories of certain gene families involved in both primary and secondary metabolism among fungi that differ in pathogenic lifestyle. We will describe our comparative structural and functional analyses, as well as our comprehensive phylogenomic treatment of all polyketide synthases (*PKS*), non-ribosomal peptide synthetases (*NPS*), ABC transporters, histidine kinases (*HK*), monofunctional catalases and kinesins from complete genomic sequence of five taxonomically diverse euascomycete fungi [the saprobe *Neurospora crassa*, the maize pathogens *Cochliobolus heterostrophus* (*Bipolaris maydis*) and *Gibberella moniliformis* (*Fusarium verticillioides*), the general cereal pathogen *G. zeae* (*F. graminearum*), and the cosmopolitan dicot pathogen *Botryotinia fuckeliana* (*Botrytis cinerea*)], and three earlier diverging ascomycetes [the hemiascomycete yeast saprobe *Saccharomyces cerevisiae*, the hemiascomycete plant pest, *Eremothecium* (*Ashbya*) *gossypii*, and the archaeascomycete yeast saprobe *Schizosaccharomyces pombe*]. Some of these data (e.g., on *PKS*s) have been used to challenge the hypothesis that small molecule products of these genes are abundant only in phytopathogenic fungi and that presence of genes for secondary metabolism reflects a history of horizontal gene transfer, much as pathogenicity islands have been transferred among pathogenic bacteria. The phylogeny of *PKS*s shows no bias in favor of plant pathogenic fungi, nor any need for horizontal gene transfer. Furthermore, *PKS*s are far more abundant than known polyketides and there are few orthologs among taxa, even between closely related species, indicating that most polyketides have yet to be characterized and that there is an enormous untapped potential for small molecule production. In contrast, data on *HK*s show that the filamentous fungi encode an extensive family of two-component signaling proteins that fall into eleven classes. Many of these groups contain *HK*s that are highly conserved among filamentous ascomycetes. Other groups are more divergent, containing gene families that have expanded within species with few clear orthologs between species. These groupings suggest that some HKs are necessary for basic functions shared by most or all ascomycetes (ie. osmosensing) while others may have evolved to adapt to specific aspects of the pathogen's lifestyle. In contrast, structural genes such as kinesin-like motor proteins have a near one-to-one correspondence of orthologs across ascomycete genomes.

Molecular adaptation in *Phytophthora*-plant interactions.

Sophien Kamoun, Jorunn Bos, Nicolas Champouret, Luis da Cunha, Shujing Dong, Elodie Gaulin, Edgar Huitema, Diane Kinney, Zhenyu Liu, Miaoying Tian, Trudy Torto. Department of Plant Pathology, The Ohio State University - OARDC, Wooster, OH.

Parasitic and pathogenic lifestyles have evolved repeatedly in eukaryotes. Several pathogenic eukaryotes represent deep phylogenetic lineages suggesting that they feature unique molecular processes for infecting their hosts. One such group is formed by the oomycetes, arguably the most devastating pathogens of dicot plants. Extensive structural genomic resources are available for the oomycete *Phytophthora* and the challenge in the post-genome era is to link sequences to phenotypes using computational tools for data mining and robust high throughput functional assays. We applied this strategy to the potato and tomato late blight pathogen *Phytophthora infestans*. Data mining for the identification of genes up-regulated during infection, encoding extracellular proteins, and undergoing diversifying selection, were combined with a variety of functional assays to identify *P. infestans* effector genes that trigger cellular and molecular responses in plant cells. The discovered effectors include: (1) The crinkler (CRN) family of general defense response elicitors; (2) Several classes of "orphan" avirulence genes that match previously unknown resistance genes; and (3) A family of extracellular protease inhibitors (EPI) that target host proteases. This research is allowing us to establish functional connections between *Phytophthora* effector genes and plant processes and to ask pertinent questions about the co-evolution of *Phytophthora* effectors with host factors.

The *Magnaporthe grisea* genome project

Ralph A. Dean and members of the International Rice Blast Consortium

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Until recently opportunities to dissect the molecular pathways governing fungal pathogenesis have been limited, in large part by lack of basic knowledge of fungal genomes. At last this is beginning to change. 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 signaling pathways of pathogenesis, including infection-related morphogenesis, and host species and cultivar specificity. In 1998, an international consortium (IRBGP) was established to sequence the rice blast genome. Initially, a BAC physical map of strain 70-15 was used a framework to create a draft sequence (~5X coverage) of chromosome 7 using the “BAC by BAC” approach coupled with a comprehensive EST program. Most recently, in collaboration with the Whitehead Institute Center for Genome Research, a shotgun approach has been undertaken to sequence and assemble the entire genome. Sequenced BAC clones, known Magnaporthe genes and ESTs were used to validate the current 6X assembly. The current status of the genome project, including annotation, comparative and functional analyses pertaining to pathogenesis, will be presented. Sequence data and other information are publicly available at the consortium website www.riceblast.org and at the Whitehead Institute

http://www-genome.wi.mit.edu/annotation/fungi/magnaporthe/.

Poster Abstracts and indexes

Biochemistry and Secondary Metabolism

1. Salt stress mediated regulation of glucose 6 phosphate dehydrogenase and glyceraldehyde 3 phosphate dehydrogenase in halotolerant *Aspergillus repens*. Phullara Shelat, Surobhi Lahri, Rajiv Vaidya and H.S. Chhatpar Department of Microbiology and Biotechnology Centre, Faculty of Science, M.S. University of Baroda, Vadodara - 390 002, Gujarat, India.

Aspergillus repens which was isolated earlier from salt pans was found to show significant growth under salinity conditions (2M). Significant increase was observed in the activities of glyceraldehyde 3 phosphate dehydrogenase and glucose 6 phosphate dehydrogenase under salt stress conditions as compared to control. Osmolytes betaine and proline did not have any influence on G6PD activity from control whereas under salt stress conditions, both these osmolytes caused reduction in enzyme activities. Glycine and glycerol however were found to decrease the activity in both control and salt stress conditions. *Asp. repens* was earlier found to accumulate Na^+ and K^+ when grown under salt stress conditions. In vitro conditions sodium and potassium were found to increase the activity of glyceraldehyde 3 Phosphate dehydrogenase, high concentrations were however found to be inhibitory. Potassium, magnesium and calcium were found to be inhibitory to glucose 6 P dehydrogenase activity from both control and salt stress conditions. Fatty acids like oleic and myristic were found to be inhibitory to glucose 6 phosphate dehydrogenase activity. Two dimensional electrophoresis showed induction and repression of some proteins under salt stress condition.

2. Yeast as a tool to study the effects of saponins on fungi. Veronika Simons and Anne Osbourn. John Innes Centre, Norwich, UK.

Saponins are antifungal secondary metabolites (glycosylated steroids, steroidal alkaloids or triterpenoids) that are associated with plant defence. Fungal pathogens of saponin-containing plants are generally insensitive to the saponins of their hosts owing to production of saponin glycosyl hydrolases or by non-degradative mechanisms. We have established *(Saccharomyces cerevisiae)* as a model to investigate the effects of saponins and to identify genes required for saponin resistance/sensitivity. Representatives of two different classes of saponins (the tomato steroidal glycoalkaloid alpha-tomatine and the oat root triterpenoid saponin avenacin A-1) inhibit the growth of *S. cerevisiae* at micromolar concentrations. The two saponins do not have identical effects on yeast. The degree of membrane permeabilization associated with inhibition of growth is substantially greater for alpha-tomatine than for avenacin A-1. Also some sterol-deficient mutants of yeast show differential sensitivity to the two saponins (for example, the erg6 mutant has reduced sensitivity towards alpha-tomatine but is hypersensitive to avenacin A-1). Importantly, the aglycones of alpha-tomatine and other steroidal alkaloids are potent growth inhibitors but this inhibition is not associated with electrolyte leakage. Analysis is being carried out using Affymetrix gene chips to assess the effects of avenacin A-1, alpha-tomatine and tomatidine on gene expression. A complete set of yeast deletion mutants is also being screened for altered saponin sensitivity. These experiments are expected to give insight into the effects of saponins on membranes and cellular processes.

3. *Aspergillus parasiticus* AFLJ interacts with AFLR and regulates transcription of aflatoxin biosynthetic pathway genes. Perng-Kuang Chang, Southern Regional Research Center, Agricultural Research Service, U. S. Department of Agriculture.

The *Aspergillus parasiticus aflJ* gene, located in the aflatoxin biosynthetic gene cluster and divergently transcribed from the aflatoxin pathway regulatory gene *aflR*, encodes a 438-amino acid protein. Disruption of *aflJ* resulted in non-pigmented mutants that lost the ability to synthesize aflatoxin intermediates. Transcript profiling by real time RT-PCR indicated that a lack of the *aflJ* transcript in the *aflJ* knockout mutants significantly decreased the transcript levels of the genes of the early (*pksA* and*nor1*), middle (*ver1*) and later (*omtA*) steps of aflatoxin biosynthetic pathway, respectively, and the reduction ranged from 5 to 20-fold. Deletion of *aflJ*, however, did not correlate with changes in the *aflR* transcript level and vice versa. Two-hybrid assays showed that AFLJ did not interact with aflatoxin biosynthetic enzymes, including NOR1, VER1, OMTA and ORDA. But AFLJ interacted with full-length AFLR, and the DNA-binding domain of AFLR was not essential for the interaction. Simultaneous substitutions of

Arg427, Arg429, and Arg431 at the carboxyl terminus of AFLR with Leu abolished its interaction with AFLJ. Substitution of Asp436, previously shown to be crucial for AFLR's activation activity, with His had little effect on the interaction. Deletions in most regions of AFLJ appeared to destroy its function despite the fact that random amino acid substitution(s) at its carboxyl terminus did not drastically affect its capacity to interact with AFLR. The results show that *aflJ* is involved in the expression of aflatoxin structural genes and support the hypothesis that *aflJ* is a coactivator gene.

4. Homologs of aflatoxin biosynthetic genes in a cluster of genes involved in the biosynthesis of a perithecial red pigment in *Nectria haematococca*. Stéphane Graziani, Christelle Vasnier and Marie-josée Daboussi. Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France

Previous work led to the identification of a polyketide synthase gene *(pksN1)*required for synthesis of a perithecial red pigment in *Nectria haematococca*. Phylogenetic analysis of this PKSN based on ketoacylacyl synthase and acyltransferase domains revealed that it belongs to the WA-type PKS which includes PKS involved in spore and mycelium pigmentation and aflatoxin pathway. The clustering of other genes with *(pksN1)*was expected in view of the gene clustering seen in secondary metabolism pathways. Subcloning and partial sequencing of the cosmid containing *(pksN1)*allowed the identification of three other ORFs with similarities to genes involved in aflatoxin (AF) and sterigmatocystin (ST) gene clusters. Their predicted activity are O-methltransferase, P450 monooxygenase and an homologue of *AflJ*. By similarity with the organisation of AF cluster which is bordered by *PKSA* and *OMTA* genes, we suspected that all or most of the genes involved in the biosynthesis of the red pigment are present in the cosmid and define a new cluster (PP for perithecial pigment). The nucleotide sequence of the genes were analyzed for matches to the AFLR regulatory protein binding motif found in *A. parasiticus* aflatoxin genes. At least, two ORFs have matching sequence to recognition sites of transcriptional factors. Further work will allow comparative studies between the two pathways.

5. Identification of beta-lactam production related ABC transporters in *Penicillium chrysogenum*. Melchior Evers[1], Hein Trip[1], Jeroen Nijland[1], Marco van den Berg[2], Roel Bovenberg[2], and Arnold Driessen[1].[1]Biological Center, Haren, The Netherlands. [2]DSM Anti-infectives, Delft, The Netherlands.

The pathway of the biosynthesis of penicillin and the cellular localization of the critical enzymatic steps has been elucidated. Little is known about the transport processes that play a role in beta-lactam excretion by *P. chrysogenum*. Since secondary metabolites are often secreted by dedicated ABC transporters, we have used a PCR-based cloning strategy to identify transporters involved in beta-lactam transport. By means of sets of degenerate primers, a set of ABC transporters were identified that are expressed under conditions of beta-lactam production. These systems were cloned and sequenced and found to belong to the family of multidrug transporters. Two of these systems are highly expressed when cells are challenged with extracellular added beta-lactams suggesting that they are involved in the secretion of beta-lactams.

6. The *CRG1* gene required for resistance to the singlet oxygen-generating cercosporin toxin in *Cercospora nicotianae*encodes a putative fungal transcription factor. Kuang-Ren Chung[1], Margaret E. Daub[2], and Christoph Schuller[3]. [1]University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850, [2]Department of Botany, North Carolina State University, Raleigh, NC 27695, [3]Institute of Medical Biochemistry, Department of Molecular Genetics, University and BioCenter Vienna, Dr. Bohr-Gasse 9/2, A-1030 Vienna, Austria

The *Cercospora nicotianae CRG1* gene is involved in cellular resistance to the perylenequinone toxin, cercosporin that generates highly toxic singlet oxygen upon exposure to light. *CRG1* gene contains an ORF of 1950 bp including a 65-bp intron. CRG1 protein with 650 amino acids contains a Cys6Zn2 binuclear cluster DNA-binding motif with homology to various fungal regulatory proteins, indicating that CRG1 may act functionally as a transcription activator. Targeted gene disruption of *CRG1* resulted in mutants that are partially sensitive to cercosporin and down-regulated in cercosporin production (<30-55%). Genetic complementation revealed that *CRG1* fully restored cercosporin resistance, but only slightly restored cercosporin production in a UV-derived mutant (CS10). Complementation of a *crg1* -null mutant, however, yielded strains that are similar to wild type in both phenotypes. These results indicated that the transcription regulator CRG1 is involved in activation of genes associated with cercosporin resistance and production in the fungus *C. nicotianae*.

7. Analysis of altered G-protein subunit accumulation in *Cryphonectria parasitica* reveals a third G-alpha homologue. Gert C. Segers, Todd B. Parsley, Donald L. Nuss and Angus L. Dawe. UMBI-CBR, 5115 Plant Sciences Bldg, College Park MD

Heterotrimeric G-proteins mediate many responses of eukaryotic cells to external stimuli and have been shown to be important for fungal pathogenicity. In this study, we explored the accumulation of G-protein subunits of the chestnut blight fungus *Cryphonectria parasitica* in mutant strains deleted for one or more putative partner subunits. Using a series of extraction buffers and immunoblot end-point dilution analysis we have established a convenient method to assess the relative abundance of these membrane-associated proteins. Disruption of either cpg-1, which encodes the G-alpha subunit CPG-1, or cpgb-1, the G-beta subunit CPGB-1, consistently reduced the level of its presumptive partner protein. This was not observed in the case of a second G-alpha subunit, CPG-2, suggesting that CPG-1 and CPGB-1 regulate each other's stability. Further, analysis of transcript levels indicated that the G-alpha and G-beta protein turnover rates were increased in the mutant strains. Additionally, a previously unidentified protein that was cross-reactive with anti-CPG-1 antiserum was found to be enhanced in liquid culture. We describe the sequence of a new G-alpha subunit, CPG-3, that is most similar to three other filamentous fungal G-alpha proteins that form a phylogenetically distinct grouping.

8. Is the aconitase from *Aspergillus nidulans* involved in the conversion of 2-methylcitrate to 2-methylisocitrate? Claudia Maerker[1], Wolfgang Buckel[2], Matthias Brock[1]. [1]Institut für Mikrobiologie Universität Hannover, Germany. [2]Laboratorium für Mikrobiologie Phillips-Universität Marburg, Germany

Most if not all filamentous fungi are able to use propionate as sole carbon and energy source. We were able to identify the pathway responsible through purification of one of the key enzymes, the 2-methylcitrate synthase from *Aspergillus nidulans* (Brock et al., 2000) and from *Aspergillus fumigatus*. 2-Methylcitrate is converted to 2-methyl-*cis*-aconitate by a specific dehydratase. Furthermore, a 2-methylisocitrate lyase was identified, which cleaves 2-methylisocitrate into succinate and pyruvate, completing the methylcitrate cycle. However, the enzyme converting 2-methyl-*cis*-aconitate into 2-methylisocitrate is still unknown. Investigations on *Escherichia coli*, which uses the same pathway have shown that this hydration is performed by the well-known citric acid cycle aconitase AcnB (Brock et al., 2002). Therfore, the first attempts at purification were made from crude extracts of *Aspergillus nidulans*, which displayed 2-methylisocitrate dehydratase activity. Analysis by SDS-PAGE showed a band of 90 kDa in all active fractions. This size is in agreement with the deduced molecular mass of an aconitase of *Aspergillus terreus*. For further analysis a cosmid library of *A. nidulans* was screened, and identified an insert, containing the entire sequence of an aconitase. Subcloning of the gene under the control of the inducible *alcA*-promotor should facilitate the purification and biochemical characterization of the encoded protein.

Literature: Brock, M., Fischer, R. Linder, D., Buckel, W. (2000) Methylcitrate synthase from *Aspergillus nidulans*: implications for propionate as an antifungal agent. Mol. Microbiol. 35, 961-973.

Brock, M., Maerker, C., Schütz, A., Völker, U., Buckel, W. (2002) Oxidation of propionate to pyruvate in *Escherichia coli*. Eur. J. Biochem. 269, 6184-94.

9. Regulation of vitamin B_2 overproduction – a stress response in *Ashbya gossypii*. Thomas Schloesser[1], Cornelia Gaetgens[1] and K.-Peter Stahmann[21]Institut für Biotechnologie 1, Forschungszentrum Juelich, Germany[2]Technische Mikrobiologie, FH Lausitz, Senftenberg, Germany

The ascomycete *Ashbya gossypii* is a natural overproducer of riboflavin (vitamin B_2). We focused on the regulation of the genes involved in biosynthesis of riboflavin. To study expression of the *RIB*-genes RT-PCR experiments were performed. Interestingly, one branch of the divided riboflavin biosynthesis pathway was found to be strongly transcriptionally regulated. Reporter studies with a *RIB3*-promoter-*lacZ* fusion showed an 8-fold increase in enzyme specific activity in the production phase. Since time courses of batch fermentations suggested a riboflavin production at low growth rates chemostatic cultivations were performed. Surprisingly, riboflavin overproduction was not detectable at constant dilution rates. This fitted with a weak expression of *RIB3-lacZ*. But, a peak of reporter expression and riboflavin overproduction was detected after down-shifts in dilution rate. Shifts from D = 0.4 to 0.02 h^{-1} resulted in an increase of reporter activity from 0.03 to 2.5 U per mg protein and an increase in riboflavin

concentration from -1. We conclude that a decline in growth rate triggers riboflavin overproduction as a stress response. This result is in line with the observation that riboflavin overproduction is linked to spore formation as a mechanism to protect the hyaline spores against UV-light.

10. The siderophore system is essential for viability of *Aspergillus nidulans:* it is the major iron uptake system and its lack causes oxidative stress. Hubertus Haas, Martin Eisendle, Ivo Zadra and Harald Oberegger Department of Molecular Biology, University of Innsbruck, Fritz-Pregl-Str. 3, A-6020 Innsbruck, Austria

The filamentous ascomycete *A. nidulans* produces three major siderophores: it excretes fusigen and triacetylfusarinine C to capture iron, and uses ferricrocin as a cellular iron storage compound. Here we report the characterization of two siderophore biosynthetic genes, *sidA* and *sidC,*encoding L-ornithine N5-monooxygenase and a nonribosomal peptide synthetase, respectively. Deletion of *sidA* resulted in a complete lack of siderophore biosynthesis. Such strains were unable to germinate and grow unless the growth medium was supplemented with siderophores. These results suggests that the siderophore system is the major iron assimilatory system of *A. nidulans.* Growth-stimulation of the siderophore-deficient mutant by a high concentration of ferrous salts suggested the presence of an additional ferrous transport system. Disruption of *sidC*resulted in the loss of the cellular siderophore ferricrocin and decreased conidiation. The intracellular labile iron pool, monitored with calcein, was significantly increased in ferricrocin-lacking mutants. Consistently, these mutants showed increased expression of genes encoding antioxidative enzymes (*sodA, catB,*and *cycA)* and elevated sensitivity to the redox cycler paraquat demonstrating that the lack of ferricrocin causes oxidative stress. Remarkably, the SidA-deficient mutant synthesized ferricrocin when feeded with triacetylfusarinine C proving that *A. nidulans* possesses the enzymatic machinery to regenerate L-hydroxyornithine from this siderophore.

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11. Characterization of *Tri16* from *Fusarium sporotrichioides* and *F. graminearum*. Nancy J. Alexander[1], Susan P. McCormick[1], Troy M. Larson[1,2] and James E. Jurgenson[31]Mycotoxin Research Unit, USDA/ARS National Center for Agricultural Utilization Research, Peoria, IL; [2]Dept. of Biol., Bradley University, Peoria, IL;[3]Dept. of Biol., University of Northern Iowa, Cedar Falls, IA

Many of the genes involved in the trichothecene biosynthetic pathway in *Fusarium* have now been identified within a 29 kb section of DNA. Within this cluster are 10 genes encoding either structural or regulatory genes of the pathway. In the search for the remaining trichothecene genes, the use of an EST library from a toxin over-producing strain carrying an altered *Tri10* has identified *Tri16*, a gene believed to be involved with trichothecene biosynthesis. We isolated and cloned this gene from *F. sporotrichioides* and *F. graminearum*, then formed disruption vectors through insertional and truncated disruption. Transformants were tested by PCR and Southern hybridization for disruption events and analyzed for toxin production. None of the disruptants showed an altered toxin phenotype. Northern analyses suggest that *Tri16* is regulated like a secondary metabolite as are several of the other toxin biosynthetic genes. *Tri16* is physically located on linkage group 2 whereas the main trichothecene cluster is on linkage group 1. Even though *Tri16* is found in the EST library, these studies show that *Tri16* is not necessary for toxin production.

12. Mycotoxin PKSs as Models for Fungal Polyketide Biosynthesis. Fengan Yu, Xiacheng Zhu, Ravi Bojja, Han Yi, Kathia Zaleta-Rivera and Liangcheng Du. Chemistry Department, University of Nebraska, Lincoln, NE 68588.

Fungi are rich sources for polyketide natural products. These products are synthesized by a group of unique polyketide synthases (PKSs), the iterative modular PKSs. Because the enzymes only have a single set of domains, it has been a mystery how the PKSs control product structural variations. We have been studying the biosynthesis of fungal polyketides by using a group of mycotoxin PKSs as a model system. These compounds have a linear carbon chain with various lengths. The single-modular PKSs, when combined, can be regarded as a ¡§heterogeneous multi-modular¡¨ system, thus, can be manipulated by the genetic approaches developed for bacterial non-iterative modular PKSs. We have made an active site mutation of the methyltransferase (MT) domain, which adds methyl groups to the polyketide carbon chain, of FUM5 from *Fusarium verticillioides*. FUM5 gene encodes a PKS catalyzing the

biosynthesis of the carbon chain of fumonisins, a group of mycotoxins that impair animal health. We have obtained several MT domain mutants and are currently working on the product identification. We have also performed a domain swapping experiment by exchanging the FUM5 ketosynthase domain with the corresponding region on PKS1 from *Cochliobolus heterotrophus*, a pathogen virulent toward Texas male sterile maize. PKS1 gene encodes a PKS responsible for the biosynthesis of T-toxins, a family of long-chain (C35 to C41) polyketides. The completion of these experiments would shed lights on the biosynthetic mechanism of fungal PKSs.

13. Characterization of a *Verticillium dahliae* hydrophobin. Anna Klimes [1] and Katherine Dobinson [1,2]. [1]University of Western Ontario, London, Ontario, Canada. [2] Agriculture and AgriFood Canada, London, Ontario, Canada.

The soil-borne fungus *Verticillium dahliae* is the causal agent of an economically significant vascular wilt disease that affects a wide range of hosts in the temperate areas of the world. The pathogen persists in the soil in the form of highly resistant resting structures known as microsclerotia. Microsclerotia serve as the primary source of disease inoculum and their formation represents a critical process in the fungal life cycle. We have identified a hydrophobin gene homologue that appears to be involved in microsclerotial development. Northern analyses indicate that the *V. dahliae* hydrophobin gene, *vdh1*, is preferentially expressed during growth at an air/solid interface. This expression pattern is consistent with the pattern of microsclerotial development *in planta*. Targetted disruption of the *vdh1* gene appears to result in a delay, or inhibition, of microsclerotial development. Studies are underway to characterize *vdh1* expression in greater detail, and to quantify the effects of *vdh1* gene disruption on microsclerotial development and on rates of disease progression in tomato plants.

14. White Collar-1, a DNA Binding Transcription Factor and a Light Sensor. Qiyang He*, Ping Cheng*, Yuhong Yang, Lixing Wang, Kevin H. Gardner, Yi Liu. UTSouthwestern Medical Center, Department of Physiology, Dallas, TX

Blue light regulates many physiological processes in fungi, but their photoreceptors are not known. In *Neurospora crassa*, all light responses depend on the Per-Arnt-Sim (PAS) domain-containing transcription factor white collar-1 (*wc-1*). By removing the WC-1 light, oxygen, or voltage domain, a specialized PAS domain that binds flavin mononucleotide in plant phototropins, we show that light responses are abolished, including light entrainment of the circadian clock. However, the WC-1-mediated dark activation of frq remains normal in this mutant, and the circadian clock can be entrained by temperature. Furthermore, we demonstrate that the purified Neurospora WC-1-WC-2 protein complex is associated with stoichiometric amounts of the chromophore flavin-adenine dinucleotide. Together, these observations suggest that WC-1 is the blue-light photoreceptor for the circadian clock and other light responses in Neurospora. * These authors contributed equally to this work.

15. Molecular cloning and genetic analysis of a symbiosis expressed gene cluster for lolitrem biosynthesis. Carolyn Young[1], Mike Christensen[2], Brian Tapper[2], Greg Bryan[2] and Barry Scott[1]. [1]Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand. [2]AgResearch, Palmerston North, New Zealand.

The indole-diterpene, lolitrem B, is well documented as the cause of 'ryegrass staggers' in animals grazing on *Neotyphodium lolii*-infected ryegrass. Although much is known about how lolitrem B is produced, the genes and proteins responsible have not been isolated. We have recently cloned a cluster of genes from *Penicillium paxilli* required for the synthesis of paxilline, an analogue of lolitrem B (Young et al. 2001) This cluster comprises a set of core genes required for indole-diterpene biosynthesis including *paxG*, a geranylgeranyl diphosphate synthase (GGPPS), *paxC*, a prenyl transferase, *paxM*, an FAD-dependent monooxygenase, and two cytochrome P450 monooxygenases, *paxP* and *paxQ*. The orthologue (*ltmG*) of *paxG* has been isolated from *N. lolii* and other related endophytes, by degenerate PCR using primers designed to conserved regions of fungal GGPP synthases. Sequence analysis of a lambda clone isolated with *ltmG* identified orthologues of *paxM* (*ltmM*) and *paxP* (*ltmP*) linked to *ltmG*. RT-PCR analysis showed that the *ltm* genes are highly expressed *in planta*. A targeted deletion of *ltmM* has been constructed in *Epichloë festucae*, and artificial associations of wild-type and the *ltmM* mutant established with perennial ryegrass. Analysis of the lolitrem phenotype of these associations has established that this gene cluster is required for lolitrem B production.

16. *dffA* gene from *Aspergillus oryzae* encodes L-ornithineN^5-oxygenase and is indispensable for deferriferrichrysin biosynthesis. Osamu Yamada[1], Suthamas Na Nan[1], Takeshi Akao[1], Mihoko Tominaga[1], Hisayuki Watanabe[2], Toshitsugu Satoh[2], Hitoshi Enei[2], and Osamu Akita[11]National Research Institute of Brewing, Hiroshima, Japan. [2]Iwate Biotechnology Research Center, Iwate, Japan.

We identified the *dffA* gene from *Aspergillus oryzae* which encodes L-ornithineN^5-oxygenase involved in the biosynthesis of deferriferrichrysin, a low-molecular-weight iron chelating compound. From more than 20,000 *A. oryzae* EST library, we found only one clone encoding a protein that exhibited homology to the *U. maydis sid1* and *P. aeruginosa pvdA* protein. The complete gene sequence shows that the *dffA* gene encodes 502 amino acids with putative FAD-binding, NADP-binding, and 'FATGY' motifs, which are conserved in *N*-hydroxylating enzymes. Northern analysis showed that this gene expression was induced under iron-limited conditions, and the promoter region has several GATA-type transcription regulator binding motifs. When the *dffA* gene was expressed under the control of the alpha-amylase promoter in *A. oryzae*, transformants revealed high L-ornithine N^5-oxygenase activities. In addition, a *dffA* gene disruptant showed no deferriferrichrysin production even under iron-limited conditions. These results suggest that the *dffA* gene is indispensable for deferriferrichrysin biosynthesis in *A. oryzae*.

17. Isolation and analyses of polyketide synthase genes from *Exserohilum monoceras*. Hisanori Tatewaki, Chihiro Tanaka, Mitsuya Tsuda. Agriculture, Kyoto University, Kyoto, Japan.

Polyketides are one of the major fungal metabolites. Some of them are well known as mycotoxins and phytotoxins, others are used for drug precursors. They are synthesized by polyketide synthase (PKS). The chemical diversity of polyketides is due to the structural diversity of PKSs. But little is known about the relation between the structure and the function of fungal PKS. Therefore, we conducted to elucidate the characteristics of PKS genes of graminicolous plant pathogenic fungi, *Exserohilum monoceras*. We have obtained at least three putative PKS genes (emp1–emp3) from *E. monoceras* by degenerate PCR. Emp1–emp3 are armatic type PKS, and especially, emp1 is closely related to PKS1 involved in melanin biosynthesis in *Colletotrichum lagenarium*. Southern blot analyses using emp1–emp3 as probe have indicated that *Bipolaris maydis* which is phylogenicaly closely related to *E. monoceras* has same set of PKS genes. We ascertained the functions of PKS genes by comparing the metabolites of wild type and PKS gene disrupted mutants. The disruptant of emp1 lacks melanin production. The chemical profiles of other disrupted mutants are now being analyzed.

18. The NADPH: cytochrome P450 reductase gene from *Gibberella fujikuroi* is essential for gibberellin biosynthesis. S. Malonek and B. Tudzynski. Institut für Botanik der Westfälischen Wilhelms-Universität Münster, Schloßgarten 3, D-48149 Münster, Germany

G. fujikuroi is famous for its production of large amounts of gibberellins (GAs). Four of the seven GA biosynthetic genes encode cytochrome P450 monooxygenases. This group of enzymes depend on NADPH: cytochrome P450 reductases (CPRs) which catalyze the transfer of electrons from NADPH via FAD and FMN to the prosthetic heme group of the P450 monoxygenase. A *cpr* gene (*cpr-Gf*) was cloned from *G. fujikuroi* that contains the conserved FAD, FMN, and NADPH-binding functional domains. Gene disruption resulted in total loss of GA production demonstrating that CPR-Gf is essential as electron donor to the four P450 monooxygenases involved in GA biosynthesis. In addition, *cpr* mutants show a reduced growth rate and are much more sensitive to benzoate due to the lost activation of another P450 monooxygenase, the detoxifying benzoate para-hydroxylase by CPR. The UV mutant *G. fujikuroi* SG138 which was shown to be blocked at most of the GA biosynthetic steps catalyzed by P450 monoxygenases, revealed the same phenotype. Sequence analysis of the mutant *cpr* allele revealed a nonsense mutation at amino acid 627. The mutant was complemented with the *cpr-Gf* and the *A. nidulans cprA* genes, and both genes restored the ability to produce GAs. Northern blot analysis revealed a co-regulated expression of the *cpr-Gf* gene and the GA biosynthetic genes under GA production conditions (nitrogen starvation). In addition, with benzoate in the medium, expression of *cpr* is induced by benzoate only, but not by nitrogen starvation anymore. These results indicate that CPR-Gf is the main but not the only electron donor for several P450 monooxygenases from primary and secondary metabolism.

19. An osmosensing histidine kinase mediates dicarboximide fungicide resistance in *Botrytis cinerea*. Wei Cui[1], Ross E. Beever[2], Stephanie L. Parkes[2] and Matthew D. Templeton[1]. [1] HortResearch, Private Bag 92169, Auckland, New Zealand. [2] Landcare Research, Private Bag 92170, Auckland, New Zealand.

A two-component histidine protein kinase (HK) gene, homologous to *os-1* from *Neurospora crassa*, was cloned and sequenced from *Botrytis cinerea* (*Botryotinia fuckeliana*) strain A1. A series of nine spontaneous laboratory mutants resistant to dicarboximide fungicides was selected from this strain and crossed to a sensitive strain. All behaved as single gene mutants and were highly osmotically sensitive but could be divided into two groups based on phenotype. Six strains showed high-level dicarboximide resistance (DafHR) and three strains showed low-level dicarboximide resistance (DafLO). Full or partial DNA sequencing of the HK gene detected single point mutations in the coiled-coil region of all nine mutants although only two of the mutants showed the same change. Genetic crosses of dicarboximide resistant isolates from the field have shown they map at a single locus, Daf1. Crosses between selected laboratory mutants and an authentic Daf1 strain showed the laboratory mutants map to this same locus. We conclude that Daf1 encodes an osmosensing HK, which mediates dicarboximide resistance, and we propose that the fungicides target this enzyme. This putative HK was fully or partially sequenced from four field dicarboximide-sensitive (DafS) strains and found to be polymorphic. Sequence studies of 27 field resistant strains (DafR) showed predicted amino acid differences from the sensitive strains in the coiled-coil region. The 27 strains could be grouped into four classes, with three classes differing from the sensitive strains by one amino acid and one class differing by two amino acids.

20. Asparaginase gene from *Aspergillus nidulans*. Tania de la Fuente, Darryl Yorkey and Patricia M. Shaffer, Department of Chemistry, University of San Diego, San Diego, CA, 92110, USA

L-asparaginase is an amidohydrolase that catalyzes the hydrolysis of asparagine to aspartic acid and ammonia. *Asparaginases* are classified into two categories, type I and type II, the latter being regulated. Aspergillus nidulans has two asparaginase genes, *apn*A (on chromosome II) and *ahr*A (on chromosome VIII). The enzyme expressed by the *ahr*A gene is categorized as a type II asparaginase [Shaffer et al. (1988) *Mol. Gen. Genet.* 212, 337-341]. Since Cereon Genomics, LLC (subsidiary of Monsanto), had sequenced the entire A.*nidulans* genome, we supplied them with the sequences of five asparaginases (type II) and received a single matching sequence (contig ANIC1307). From this sequence we prepared PCR primers and will use them with genomic DNA to produce a nucleotide sequence containing one of the L-asparaginase genes. We will clone this sequence into a vector in order both to amplify the DNA for sequencing and express it for enzymatic testing of this possible asparaginase gene (*apn*A or*ahr*A). Since type II asparaginases are used as a cure for childhood acute lymphoblastic leukemia, this research may have some pharmaceutical significance.

21. A global regulator of secondary metabolism in *Aspergillus*. Jin Woo Bok and Nancy P. Keller Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Dr., Madison, WI 53706

Secondary metabolites are low molecular weight natural products that are not essential to the producing cells but likely have a survival function in nature. They are of intense interest to humankind due to their pharmaceutical and/or toxic properties. We have identified a novel*Aspergillus nidulans* protein, LaeA, which regulates gene expression of several secondary metabolites. This protein is conserved in *A. fumigatus* and likely other ascomycetes. LaeA is a nuclear located protein that positively regulates sterigmatocystin biosynthesis, mycelial pigment formation, penicillin biosynthesis, lovastatin biosynthesis and asexual spore pigment biosynthesis in *A. nidulans*, and mycelial pigment formation, asexual spore pigment biosynthesis and gliotoxin biosynthesis in *A. fumigatus*. mRNA studies of both delta*laeA* and over expression *laeA* strains suggest this regulation is transcriptional. Two signal transduction molecules (protein kinase A and rasA) which negatively regulate sterigmatocystin biosynthesis and asexual sporulation in *A. nidulans* also negatively regulate *laeA* expression. However delta *laeA* strains show little difference in asexual spore production from wild type thus suggesting the primary role of LaeA is to regulate secondary metabolism. Current studies are aimed at deciphering the mechanism of this regulation.

22. Reconciliation of the genetically defined *Fum3* locus with the molecularly defined*FUM9* gene in *Gibberella moniliformis*. Robert A. E. Butchko, Ronald D. Plattner, and Robert H. Proctor. National Center for Agricultural Resource Utilization, ARS, USDA, 1815 N. University St., Peoria, IL 61604.

Gibberella moniliformis causes ear and stalk rot of maize and can produce the polyketide-derived mycotoxins fumonisin B_1, B_2, B_3 and B_4. Fumonisins disrupt sphingolipid biosynthesis in animal cells, cause leukoencephalomalacia and pulmonary edema in horses and swine respectively, and are associated with liver and kidney cancer in laboratory rodents. A fumonisin biosynthetic gene (*FUM*) cluster, consisting of 15 co-regulated

genes, was recently described in *G. moniliformis*. BLAST comparisons indicate that most genes in the cluster are likely to encode proteins involved in fumonisin biosynthetic reactions. During our ongoing deletion analysis of *FUM* genes, we have been able to determine the molecular basis of some naturally occurring and induced *G. moniliformis* mutants defective in fumonisin production. Here, we describe evidence for the molecular basis of one of these mutations at the genetically defined *Fum3* locus. Deletion mutants of *FUM9*, a cluster gene predicted to encode a dioxygenase, produce only fumonisins B3 and B4, which lack a hydroxyl group at carbon 5. This is the same phenotype exhibited by strains with mutations in the *Fum3* locus. We sequenced the region corresponding to *FUM9* in a*Fum3* mutant and identified a transition mutation that introduces a stop codon early in the*FUM9* protein-coding region. Together the results indicate that the *FUM9* gene is equivalent to the *Fum3* locus. Currently, we are attempting to complement the*Fum3* mutant by transformation with a wild-type *FUM9* allele.

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

The macrolide antibiotics bafilomycin and concanamycin are potent inhibitors of V- ATPases. To identify the binding site of bafilomycin we selected mutant strains of *Neurospora crassa* (named *bfr*) that are resistant to this antibiotic. In one class of *bfr* strains the V-ATPase was resistant to inhibition in vitro. These strains had five different point mutations in the *vma-3* gene, which encodes the hydrophobic c subunit of the vacuolar ATPase. Two mutations were in the region between the 1st and 2nd transmembrane helix. The other three sites were in the 4th membrane helix, near the putative proton-binding site (glu-138). Thus, the mutated sites appear to be on the outer face of the "rotor" sector of the enzyme, a region hypothesized to form an interface with the "a" subunit.

Surprisingly, the *bfr* strains had little resistance to concanamycin, which has a similar structure. By further mutagenizing one of the *bfr* strains we obtained four new strains that were resistant to both antibiotics. Each of these had two altered residues in the c subunit. Thus, concanamycin does appear to bind to the same region, but we have been unable to obtain a concanamycin-resistant strain that has only one altered residue.

The positions of two of the mutated residues in the *bfr* strains correspond precisely to the positions of mutated residues in the homologous c subunit of the mitochondrial ATPase that confer resistance to oligomycin. These results suggest that vacuolar and mitochondrial ATPases have an ancient, conserved antibiotic binding site. As the sequences of the polypeptides have diverged, new antibiotics that target the same vulnerable site in this family of enzymes have arisen. The data also provide support for the hypothesis that the tertiary structure of the c subunit of the V-ATPase is very similar to that of the c subunit of the F-ATPase.

24. Functional variation of *Tri8* in *Gibberella zeae*. Seung Hoon Lee[1], Theresa Lee[1], Sung-Hwan Yun[2], and Yin-Won Lee[1]. [1]School of Agricultural Biotechnology, Seoul National University, Suwon, 441-744, Korea. [2]Division of Life Sciences, Soonchunhyang University, Asan, 336-745, Korea

Gibberella zeae, a cereal head blight fungus, produces sesquiterpene epoxides, 8-ketotrichothecenes such as deoxynivalenol (DON) and nivalenol (NIV). In most cases, this fungus produces acetylated derivatives along with the 8-ketotrichothecenes. The DON chemotype isolates co-produces 3-acetyl-DON (3-ADON) or 15-acetyldeoxynivalenol (15-ADON) with DON and the NIV chemotype co-produces 4-acetyl-NIV (4-ANIV) with NIV. Molecular studies have so far revealed that *Tri8* and *Tri7*, located at a gene cluster of *G. zeae*, were responsible for production of these acetylated compounds:*Tri8* for deacetylation at C-3 in a 15-ADON-producing strain and *Tri7* for acetylation at C-4 in 4-ANIV-producing isolates. In this study, we have focused on the*Tri8* genes as a possible genetic element for structural variation at the C-3 position of 8-ketotrichothecenes produced by *G. zeae*. First, we have compared amino acid sequences of *Tri8* from three Korean strains. Sequence analyses, however, showed that SCD2, 3-ADON-producing strain, carried an intact copy of *Tri8* open reading frame (ORF) as 15-ADON-producing (H-11) and NIV-producing (88-1) strains, both lacking the acetyl group at the C-3 position. To prove the role of *Tri8*, an internal region of *Tri8*ORF was disrupted in each strain. Transgenic H-11 strains created by disruption of *Tri8* accumulated 3, 15-ADON rather than 15-ADON in solid culture as previously reported. However, disruption of *Tri8* in both SCD2 and 88-1 caused no alteration in trichothecene biosynthesis. These results suggest that, unlike the H-11 Tri8, the SCD2 Tri8 protein lacks the ability to remove the acetyl group at the C-3 in trichothecene

biosynthesis. In addition, the result that the 88-1 Tri8 is dispensable may suggest other pathway for deacetylation of the C-3 in NIV chemotype of *G. zeae* isolates. Further functional studies to support these hypotheses are in progress.

25. Pathways for synthesis of polyunsaturated fatty acids in the oleaginous Zygomycete *Mortierella alpina*. Chris Lounds, Adrian Watson, Marcos Alcocer, Andrew Carter*, Donald MacKenzie* and David Archer School of Life and Environmental Science, University of Nottingham, Nottingham NG7 2RD, UK. *Institute of Food Research, Norwich NR4 7UA, UK.

Linoleic acid (C18:2,n-6) and alpha-linolenic acid (C18:3,n-3) are essential components of the mammalian diet. Humans lack the ability to produce these PUFAs *de novo* due to a lack of key fatty acid desaturases (specifically the delta-12 and delta-15). These essential fatty acids are elongated and undergo further desaturations to produce a range of n-6 and n-3 polyunsaturated fatty acids (PUFAs) including arachidonic acid (C20:4,n-6). The supply of arachidonic acid can be limiting, for example in babies and infants, so some baby milk formulations are supplemented with arachidonic acid. The Zygomycete *Mortierella alpina* produces up to 50% of its dry weight as oil, of which 40% can be arachidonic acid, providing a source for supplementation of formula milk.

We have developed genetic transformation systems in two strains of *M. alpina* in order to be able to study the regulation of the pathways of oil accumulation and to manipulate the metabolic pathway of PUFA biosynthesis. We have also studied the impact of culture conditions on the yield and type of PUFAs synthesised. High C, N-limited media favour the accumulation of oils and a range of transcripts have been monitored to determine which genes are likely to be regulated transcriptionally under these conditions. Two of the three fatty acid delta-9 desaturase genes identified in this fungus, for example, are shown to be transcriptionally regulated by high carbon to nitrogen levels. In addition to the regulation of individual genes by nutrients we are examining the role of epigenetic mechanisms in transcriptional regulation in *M. alpina* and have shown that *M. alpina* has methylated DNA.

26. Biosynthesis of sulfur amino-acids in *Magnaporthe grisea*: molecular and biochemical characterization of cystathionine gamma-lyase. Audrey Beaurepaire[1], Pascale Balhadhère[2], Nick Talbot[2], Marc-Henri Lebrun[1], Michel Droux[1]. [1]1932 CNRS-Bayer CropScience, Lyon, France. [2]University of Exeter, Exeter, United Kingdom

Mutant characterization in *N. crassa* and *E. nidulans* indicates that sulfur assimilation involves different pathways than those found either in plants or in the yeast *S. cerevisae*. Using the recently released draft genome sequence of the rice blast fungus (*M. grisea*), we identified most of the genes involved in sulfate assimilation and in cysteine/methionine biosynthesis. Biosynthesis of sulfur amino-acids is essential for the pathogenic development of *M. grisea*. Our goal is to understand at a molecular and biochemical level, the role of sulfur assimilation in the development of the fungus during plant infection, and to compare this to sulfur metabolism in the host plant. We have characterised cystathionine gamma-lyase, which is involved in the reversed transsulfuration sequence from homocysteine to cysteine (the *S. cerevisae* homologue). The corresponding cDNA was over-expressed in recombinant bacteria and the resulting protein purified to homogeneity. Biochemical characterization of the proteins will be presented and the data were compared to enzymes of this family catalyzing a similar reaction.

27. Screening of *Fusarium graminearum* mutants for loss of zearalenone production using yeast bioassays. Naser Safaie[1,2], Michaela Peruci[1], Herwig Bachmann[1], Rudolf Mitterbauer[1], Frances Trail[3] and Gerhard Adam[1]. [1]Center of Applied Genetics, University of Agricultural Sciences, Vienna, Austria. [2]Tarbiat Modarres University, Tehran, Iran (present address),[3]Michigan State University, MI, USA.

Zearalenone (ZON) is a mycotoxin produced by several species of *Fusarium* , which is problematic due to its xeno-hormone activity in animals and humans. As plants do not possess an estrogen receptor, the role of ZON and its derivatives in the plant-pathogen interaction is unknown. We have developed yeast bioassays that allow cost effective detection and quantification of the estrogenic activity present in biological samples. Growth of strain YZRM7 is only possible, when ZON is present in the yeast medium in concentrations higher than 1 ppb (microgram/kg). We have also constructed an ABC transporter deficient strain (YZHB817) expressing a hybrid transcription activator consisting of the DNA binding domain of the yeast Gal4p and the hormone dependent activation domain of the human estrogen receptor, allowing quantitative determination of 5-100 ppb ZON with a *GAL7-lacZ* reporter gene. Using these two assays we have screened 1000 insertional mutants of *F. graminearum*.

Although the wild type strain PH-1 produces low amounts of ZON on plates, we could easily detect it in agar plugs. Several promising candidates producing very little or undetectable amounts of ZON when grown on plates or on liquid starch glutamate medium have been identified. The mutants should be valuable tools for identification of ZON biosynthetic genes and for virulence testing.

28. Peroxisomal Origins of Aflatoxin/Sterigmatocystin Biosynthesis. Lori A. Maggio-Hall, Richard A. Wilson and Nancy P. Keller Department of Plant Pathology, University of Wisconsin-Madison

The *Aspergillus* mycotoxins, aflatoxin (AF) and its precursor sterigmatocystin (ST), are carcinogenic polyketides assembled from acetyl-coenzyme A units. We are interested in how the acetyl-CoA pool is controlled and made available for secondary metabolic pathways, particularly the AF/ST pathway. One important cellular mechanism for controlling this pool is its compartmentalization into different organelles. Here we present evidence that one of these organelles, the peroxisome, plays a role in the synthesis of ST in *A. nidulans*. We have found that a mutant lacking the delta-12 desaturase gene (*odeA*) shows signs of peroxisomal proliferation, accumulates oleic acid, overexpresses beta-oxidation and ST biosynthetic genes and makes substantially more ST than a wild type strain. Growth medium containing oleic acid supports a similar phenotype in the wild type strain. Fatty acid beta-oxidation is the major endogenous source of acetyl-CoA units in this organelle. Disruption of *fox2*, the gene encoding the multifunctional beta-oxidation protein of *A. nidulans*, results in a strain unable to grow on oleic acid as the sole carbon and energy source and crippled in the ability to synthesize ST. However, expression levels of AF/ST biosynthetic (*stc*) genes were not affected by the mutation. Finally, microscopic studies showed that the AF/ST precursor norsolorinic acid (NOR) appears to accumulate in the peroxisomes of NOR-accumulating mutant strains. Since NOR is the first stable intermediate after the polyketide synthase step in the pathway, it is possible that acetyl-CoA-requiring steps occur in the peroxisome. We are currently exploring the subcellular localization of these steps which require the polyketide synthase (StcA) and fatty acid synthase (StcJK), and analyzing the effect of mutations that alter carbon flow into and/or out of the peroxisome on the synthesis of ST.

29. Non-ribosomally synthesized peptides in *Fusarium culmorum*. Carsten T. Tobiasen and Henriette Giese Institute of Ecology, Section of Genetics, Royal Veterinary and Agricultural University (KVL). Thorvaldsensvej 40, 1870 Frb. C, DK.

Fusarium culmorum is the most frequent *Fusarium* species in Danish soils and is believed to be the major cause of *Fusarium* head blight of barley in Denmark. Peptides of non-ribosomally origin have been reported to be important in plant pathogenesis. An example of such a peptide is enniatin (Burmeister and Plattner, 1987), which consist of different analogs and is one of the most well-described non-ribosomally synthesized peptides (NRSP). Enniatin production has been reported by many different *Fusarium* spp., but *F. culmorum* do not synthesize enniatin. To obtain an idea of the peptides present in *F. culmorum* a chemical purification is performed. The initial screening was done by HPLC and one peak of interest was selected for further characterization. The compound were purified on a Sephadex LH20 column and by preparative HPLC, while LC-MS and NMR will be used for structure elucidation. To identify NRPS genes in *F. culmorum* we apply two different approaches. A modified gene specific differential display technique is used as well as screens of cDNA libraries that will compliment the DD-technique. Fragments of an adenylation- and condensationdomain have already been cloned and are used for optimal primer design and as probes for the library screens. These approaches will help us identifying the final module of the NRPS gene and facilitate the identification of the encoded peptide.

Ref.: Burmeister H.R. and Plattner R.D. (1987). Enniatin production by *Fusarium tricinctum* and its effect on germinating wheat seeds. Physiology and Biochemistry, Vol. 77 (10) p. 1483-1487.

30. Investigation of the pentose phosphate pathway in *Trichoderma reesei*: disruption of the phosphoglucose isomerase gene. M. Carmen Limón, Jaana Uusitalo, Tiina Pakula, Markku Saloheimo and Merja Penttilä. VTT Biotechnology, P.O. Box 1500, FIN-02044 VTT, Finland.

T. reesei is widely used for industrial protein production, very little is however known about its physiology and primary metabolism. In order to study the role and strength of the *T. reesei* pentose phosphate pathway (PPP), we have generated a phosphoglucose isomerase (PGII) disruptant in the strain RutC30. The disruption of this gene

blocks glycolysis at the second reaction step from glucose-6-P to fructose-6-P and directs the major carbon flux to PPP. The gene disruptants show a clearly different phenotype from the parental strain. They do not grow with fructose, glycerol or xylose as the sole carbon source but growth is restored if glucose is added to the media. This indicates that glucose is needed in the cells for e.g. lipid and cell wall component synthesis. When glucose is the only carbon source, the disruptants display small colonies on plates and pellets in liquid media but they are able to grow at different concentrations of glucose. Moreover, the disruptants have an altered morphology. Glycolytic enzymes such as pyruvate kinase had lower activity in the *pgi1* disruptants than in the parental strain. On the other hand, glucose-6P-dehydrogenase that directs glucose to PPP had somewhat higher activity in the disruptants. The results of this study indicate that *T. reesei* has a relatively active pentose phosphate pathway and in this respect it resembles more *Kluyveromyces lactis* than *S. cerevisiae*.

31. *veA* is necessary for normal secondary metabolism in *Aspergillus nidulans*. Kato N, Brooks W and Calvo AM. Biological Sciences, Northern Illinois University, DeKalb, IL

Aspergillus spp. mycotoxins affect corn, peanuts, cotton, sorghum and tree nuts. Controlling mycotoxin biosynthesis or fungal dissemination could eliminate impact on health and the economy. Because signaling pathways tend to be conserved in *Aspergillus* spp., the model system *Aspergillus nidulans* is used to study regulation of mycotoxin biosynthesis and development. Most studies on regulation of morphological differentiation in*Aspergillus nidulans* focus on conidiation. Some pathways regulating asexual development also regulate mycotoxin biosynthesis. *Aspergillus* spp. also produce resistant structures: fruiting bodies called cleistothecia in *Aspergillus nidulans* or sclerotia in *Aspergillus flavus* and *Aspergillus parasiticus*. Said structures allow survival in adverse conditions. Because it is posited that sclerotia derive from cleistothecia, it is likely that conserved signaling pathways controlling cleistothecial development also control sclerotial formation in *Aspergillus flavus* and *Aspergillus parasiticus*, the major aflatoxin producers. Molecular studies on control of cleistothecial or sclerotial development are limited. Few regulatory genes have been identified in *Aspergillus nidulans*. Deletion of one of these genes, *veA*, blocks cleistothecial production. We found that *veA*deletion alters secondary metabolism, preventing synthesis of the mycotoxin sterigmatocystin and of penicillin. We identified a *veA* homolog in *Aspergillus parasiticus* and we are characterizing its function.

32. Investigating the acquisition of HC-toxin-resistant histone deacetylase activity in the maize pathogen *Cochliobolus carbonum*. Pierre-Henri Clergeot[1], Jennifer A. Bieszke[1], Dipnath Baidyaroy[2] and Jonathan D. Walton[1].[1]MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA. [2]Current address: Dow Chemical Company, San Diego, CA 92121, USA.

HC-toxin, a cyclic peptide produced by the filamentous ascomycete *C. carbonum*, is a major determinant of pathogenicity towards maize lines homozygous recessive at the *HM1* locus. *In vitro*, it is a broad spectrum inhibitor of histone deacetylases (HDACs) in plants, animals, and fungi. With age, still cultures of *C. carbonum* acquire HC-toxin-resistant HDAC activity (Brosch *et al.*, 2001, Biochemistry 40:12855). Genetic evidence shows that acquisition of resistance is independent of toxin biosynthesis, but linked to the TOX2 locus (Baidyaroy *et al.*, 2002, Eukaryotic Cell 1:538). Furthermore, resistance was found to be independent of three of the four HDAC genes from *C. carbonum*, *HDC1*, *HDC3* and *HDC4*. Fractionation of both HC-toxin-sensitive and resistant crude HDAC extracts by gel filtration showed that acquisition of resistance correlates with a shift of HDAC activity from high to low molecular weight proteins. Western blotting shows that resistance correlates with a structural modification of Hdc2 to a form that is no longer recognized by an antibody raised against its C-terminus. Both HC-toxin-resistant HDAC activity and structural modification of Hdc2 can be gained by a sensitive crude HDAC extract upon mixing with a resistant one. These results are consistent with resistance being due to a post-translational modification of Hdc2. Making the hypothesis that the processing of Hdc2 might be critical for the self-protection of *C. carbonum* against its own toxin, we are currently working to isolate the putative processing factor or factors.

33. Gene Clusters Associated with Production of 1-aminopyrrolizidine (Loline) Alkaloids in the Grass Endophyte *Neotyphodium uncinatum*. Spiering, Martin J., Moon, Christina D., and Schardl, Christopher L. Department of Plant Pathology, University of Kentucky, Lexington, Kentucky

Fungi of the genus *Epichloë* (anamorphs, *Neotyphodium*) are endophytic symbionts of grasses, frequently promoting grass persistence. In certain*Epichloë/Neotyphodium*--grass associations, insecticidal 1-aminopyrrolizidine (loline)

alkaloids are produced, which hold promise as natural plant protectants. The objective of our research is to identify genes involved in loline production. Several genes differentially expressed during loline accumulation in *N. uncinatum* cultures were isolated by suppression subtractive hybridization. Two genes isolated, *lolA* and *lolC*, had similarity to genes encoding aspartate kinases and homocysteine synthase, respectively, enzymes in methionine biosynthesis. *lolA* and *lolC* were highly expressed during loline production in culture, and were present only in endophytes with a loline-producing phenotype. Long PCR indicated linkage of *lolA* and *lolC* in the *N. uncinatum* genome, and genome walking showed that *lolA* and *lolC* are part of a cluster of 10 putative genes in a genomic region of 24 kb. Moreover, *N. uncinatum* has two allelic *lol* clusters, and expression of six of these genes in loline-producing *N. uncinatum* cultures was confirmed. The putative *lol* genes are related to known genes for monooxygenases, oxidoreductases, epoxidases, and pyridoxal phosphate-containing enzymes. Many of these genes related to the putative *lol* genes are also present in secondary-metabolite gene clusters of fungi. Gene-disruption experiments are now underway to test involvement of the gene cluster in loline alkaloid production.

34. Evolution of a secondary metabolite gene cluster implicated in loline alkaloid biosynthesis of grass-endophytes (*Epichloë* and *Neotyphodium* spp.). Kutil, Brandi L., Martin J. Spiering, Christopher L. Schardl and Heather H. Wilkinson. Plant Pathology, Texas A&M University, College Station, TX. Plant Pathology, University of Kentucky, Lexington, KY.

Assembly of a novel fungal secondary metabolite gene cluster likely involves recruitment of genes from other dispensable pathways and/or duplication and recruitment of genes from non-dispensable primary metabolism pathways. Fungal loline alkaloid (saturated 1-aminopyrrolizidine alkaloids with an oxygen bridge) production is associated exclusively with the closely related grass-endophyte species in the genera *Epichloë* and *Neotyphodium*. Recent identification and partial sequencing of gene clusters associated with lolines production (*LOL*) in *Epichloë festucae* and *Neotyphodium uncinatum* has revealed at least 7 ORFs implicated in the trait. *N. uncinatum* has two separate clusters with duplicates of some of the ORFs present at both loci. There is a high degree of microsynteny (gene order and orientation) among the *E. festucae* and *N. uncinatum* clusters. The ORFs show close homology to either genes normally associated with fungal secondary metabolism (e.g. cytochrome P450, oxidoreductase) or genes involved in polyamine biosynthesis (e.g. ornithine decarboxylase, homocysteine synthase). To address the evolutionary origins of the cluster we are investigating the phylogenetic relationships of particular *lol* ORFs in *E. festucae* to both orthologous genes in lolines expressing endophyte species and to paralogous genes (or gene families) in *E. festucae*, other endophytes, and model ascomycetes. Additionally, to determine whether there is evidence for seeding of the cluster with recruitment and/or duplication of linked genes, we are investigating the linkage of these same *lol* paralogs. While it is clear that these insecticidal compounds should convey a selective advantage, thus explaining maintenance of the trait, this analysis should provide a glimpse into the events that led to their origin.

35. *Agrobacterium tumefaciens*-mediated transformation of *Mycosphaerella fijiensis*, the black Sigatoka pathogen of bananas. Bruno Giuliano Garisto Donzelli and Alice C.L. Churchill, Boyce Thompson Institute for Plant Research at Cornell University, Ithaca, NY, 14853-1801, USA

Bananas and plantains (*Musa* sp.) are among the world's most important agricultural products, ranking fourth after rice, wheat, and maize for gross value of production. Black Sigatoka disease, caused by *Mycosphaerella fijiensis*, is currently the most destructive and economically important disease of *Musa* sp. worldwide. The disease is controlled in commercial plantations through the application of fungicides up to 45 times per growing season. Little is known of the molecular interactions between the pathogen and its *Musa* hosts. We have developed a method for *Agrobacterium tumefaciens*-mediated transformation (ATMT) of *M. fijiensis* to facilitate efficient genetic manipulations of this fungus. Transformations were carried out utilizing a mixture of spores and hyphal fragments as recipients for binary vectors, which carried hygromycin phosphotransferase as the selectable marker, as well as a gene for green fluorescent protein (GFP) expression in some cases. We obtained yields of up to 90 hygromycin-resistant colonies/plate after co-cultivation of the fungus with *A. tumefaciens*; transformants expressing GFP constitutively were also obtained. Most of the transformants resulted from single copy T-DNA insertions. Melanin shunt metabolites and other fungal toxins have been proposed to play a role in the disease by causing extensive leaf tissue necrosis. We have cloned fragments of several genes potentially involved in toxin production, including three genes encoding polyketide synthases (PKS), one THN-reductase, one alcohol oxidase, two alcohol dehydrogenases, and a transport facilitator protein. Molecular characterization of a putative melanin-type PKS gene by targeted gene

disruption is in progress. Additionally, we have developed analytical HPLC methods to characterize changes in metabolic profiles of mutants of *M. fijiensis*.

36. The molecular profiles of low molecular metalloprotease from *A. fumigatus*. Youhei Yamagata, Takesi Kawamura, Ichiro Imao, Megumi Ose, Ohnishi Fumito, Keietsu Abe, Tasuku Nakajima. Agriculture, Tohoku Univ. Sendai, Japan.

Aspergillus fumigatus is one of the aspergillosis-causing fungi. The fungus produces some extracellular proteolytic enzyme, such as a serine protease and a metalloprotease. It has been thought that the proteolytic enzymes play important roles during early infection. Elastin occupies about 28% of lung proteins and it was reported that the elastinolytic activity was essential for infectious capacity of *A. fumigatus*. The serine protease has elastin degradation activity. Thermolysin like high molecular weight metalloprotease can digest collagen and gelatin but cannot hydrolyze elastin. The defective mutant of two proteases, however, maintaines infectious capacity and the expression level of the low molecular weight metalloprotease (MEP20) becomes higher than that of the wild type. MEP20 has been thought to be concerned with elastin digestion. We cloned the gene encoding a low molecular weight metalloprotease of *A. fumigatus*from Japanese aspergillosis patient and the enzyme was expressed by using *A. oryzae* as a host. We compared the amino acid sequences of MEP20s of *A. fumigatus* from Japan, America and Europe. The identities of amino acid sequences were shown only 87%, 59% and 56% between the enzymes from Japanese and American strains, Japanese and European strains, and American and European strains, respectively. The identity between the amino acid sequences of MEP20 from Japanese *A. fumigatus* and deutelolysin from Japanese fermentative strain *A. oryzae* was also only 60%. Amino acid sequences of MEP20s showed the diversification of depending on the location in *A. fumigatus*. We will discuss the enzymatic profiles of low molecular weight metalloproteases.

37. A 14-3-3 Homolog in *Aspergillus flavus* affects Aflatoxin Production. Ahmad M. Fakhoury and Gary A. Payne Department of Plant Pathology North Carolina State University

The filamentous fungus, *Aspergillus flavus*, is a pathogen of several crops including corn, cotton, peanuts and tree nuts. Upon infection, the fungus produces aflatoxins, a group of secondary metabolites known to cause cancer in animals. The biosynthesis of aflatoxin is influenced by environmental factors such as temperature, pH, carbon and nitrogen sources. A thorough understanding of the regulation of aflatoxin biosynthesis has proven to be a difficult task given the diversity of the cues involved. We identified a 14-3-3 homolog in an EST library made during aflatoxin biosynthesis. This class of proteins is thought to act as a switch coordinating the allocation of metabolites among different metabolic pathways. 14-3-3 proteins are ubiquitous in their distribution with functions ranging from regulating primary metabolism in plants, to controlling trafficking in cells. To determine if 14-3-3 plays a role in aflatoxin biosynthesis we disrupted the gene in *A. flavus* strain 86-10 by site-directed mutagenesis. Disruption of the gene in *A. flavus* resulted in subtle morphological changes in the generated mutant in comparison with the parent strain. These included a decrease in the rate of growth and in conidial germination and an increase in the sensitivity to temperature. Most interestingly, the strain with the disrupted 14-3-3 gene did not produce aflatoxin under several tested conditions. Complementing the mutation with a functional 14-3-3 gene rescued the ability of the fungus to produce aflatoxin.

38. Polyamines in *Phytophthora sojae*-Soybean Interactions. Marcus Chibucos & Paul Morris. Bowling Green State University, Bowling Green, OH USA.

Because zoospores are the predominant dispersal mechanism of the plant pathogenic oomycete *Phytophthora sojae*, an understanding of zoospore biology may contribute to new control mechanisms. In order to facilitate study of gene expression in swimming zoospores, cDNA was generated and PCR-amplified using different primers. A primer designed to amplify an ABC transporter yielded, due to mis-priming, a reproducible 1.5 kb product. A TBLASTX search at NCBI revealed 56% sequence identity and 67% sequence homology at the amino acid level to a putrescine transporter in *Pseudomonas aeruginosa*. High-stringency Southern analysis of an EcoRI-digested BAC clone with ^{32}P-labeled 1.5 kb product revealed 5 kb and 10 kb fragments. Fragments were cloned into pUC18, transposed with an EZ::TN^{TM} Kit (Epicentre) and sequenced. Because the putative transporter was identified in cDNA, suggesting functional expression, active putrescine assimilation was assessed in swimming zoospores. 1,4-^{14}C-putrescine uptake experiments demonstrated constitutive transporter expression with K_M of 2 micromolar. Additional

experiments have indicated that vegetative hyphae can utilize di- and polyamines as a sole nitrogen source. Demonstration of polyamine uptake by zoospores and hyphae, coupled with previous detection of soil polyamines, suggests importance of polyamines in rhizosphere-pathogen dynamics. Polyamine exudation by roots was confirmed by benzoylation of root secretions, followed by chromatographic separation by HPLC. Future research will explore the role of polyamines in *P. sojae*-soybean interactions.

39. Metabolic modeling of acetyl-CoA mutants of *Aspergillus nidulans.* Jessica H. Marshall, Harvey W. Blanch and Jay D. Keasling Department of Chemical Engineering, University of California, Berkeley, CA

Aspergillus species produce many secondary metabolites including the toxic, carcinogenic polyketide compounds aflatoxin (AF) and its precursor sterigmatocystin (ST). AF and ST are synthesized from acetyl-CoA. Mutations in genes associated with acetyl-CoA metabolism can dramatically affect the amount of ST produced in *A. nidulans* (N. Keller, personal communication). We are using metabolic modeling techniques, specifically metabolic flux and isotopomer analyses, to characterize the flow of carbon through primary and secondary metabolism in these mutant *A. nidulans* strains. Metabolic flux analysis calculates the metabolic fluxes through all reactions included in an organism's metabolic network, and thus allows quantification of the effect of genetic manipulations or changes to growth conditions on the entire metabolic network. In isotopomer analysis, ^{13}C-labeled glucose is fed to the cells, and the label pattern of metabolites such as amino acids is measured and used as model input to improve flux calculations.

Here we present flux calculations obtained from our model using measurements of steady-state biomass composition and amino acid ^{13}C label distribution from continuous cultures of *A. nidulans* as inputs.

40. Trichothecene biosynthesis by *Fusarium sporotrichioides* requires a second biosynthetic gene cluster. Daren W. Brown, Robert H. Proctor and Ronald D. Plattner. Mycotoxin Research Unit, NCAUR, USDA/ARS, 1815 N. University St., Peoria, IL 61604

Fusarium species produce a variety of toxic trichothecenes including T-2 toxin, deoxynivalenol (DON) and nivalenol (NIV). These toxins are potent inhibitors of protein synthesis and are an agricultural problem due to their detrimental affect on human and animal health. For some *Fusarium* species, trichothecenes are a critical component of virulence on certain crop plants. Almost all of the genes so far characterized involved in trichothecene biosynthesis in *F. sporotrichioides* and *F. graminearum* are located in a cluster comprised of 10 to 12 open reading frames (ORFs). TRI101 is unlinked to the core cluster and is flanked by house-keeping genes. At present, the biochemical pathway leading from farnesyl pyrophosphate to T-2 toxin in *F. sporotrichioides* includes 15 steps of which 8 have been assigned to specific genes. This report describes the characterization of two new genes that are required for trichothecene biosynthesis. These two adjacent genes are not located near the previously characterized trichothecene genes and are flanked by ORFs that do not appear to be involved in trichothecene biosynthesis. These two genes represent a second, mini-cluster that is required for toxin synthesis.

41. Alterations in B versus C fumonisin production by transformation of the *Fusarium oxysporum FUM8* coding region into *Gibberella moniliformis*. R.H.Proctor[1], R.D. Plattner[1], J.-A. Seo[1,2] and Y.-W. Lee[2]. [1]USDA ARS National Center for Agricultural Utilization Research, Peoria, Illinois and [2]Seoul National University, Suwon, Korea.

Fumonisins are carcinogenic mycotoxins produced by the maize pathogen *Gibberella moniliformis* (anamorph *Fusarium verticillioides*), several closely related Fusarium species, and at least one isolate of *F. oxysporum*. *G. moniliformis* produces predominantly B fumonisins, which are likely formed via the condensation of alanine and a 20-carbon polyketide. In contrast, the isolate of *F. oxysporum* produces predominantly C fumonisins, which are likely formed via the condensation of glycine and the same 20-carbon polyketide. The *FUM8* gene is required for fumonisin production and its predicted protein is highly similar to the yeast sphingolipid biosynthetic enzyme that catalyzes the condensation of serine and palmitic acid. This similarity suggests the *FUM8* protein catalyzes the condensation of alanine, or glycine, and the polyketide during fumonisin biosynthesis. To determine whether *FUM8* is responsible for the different fumonisin production profiles of *G. moniliformis* and *F. oxysporum*, we constructed a hybrid gene consisting of the *G. moniliformis FUM8* promoter region fused to the *F. oxysporum FUM8* coding

region. Transformation of this hybrid gene into *G. moniliformis* changed the fumonisin profile from predominantly B fumonisins to predominantly C fumonisins when the hybrid gene integrated at *FUM8*. In contrast, only slight changes in the fumonisin profile occurred when the hybrid gene integrated elsewhere. These results indicate that *FUM8* is responsible for B versus C fumonisin production in*Fusarium/Gibberella*.

42. A Fungal Specific Protein Domain Senses Arginine For Coordinate Feedback Inhibition of Two Enzymes. Catherine A. McKinstry and Richard L. Weiss. Department of Biochemistry UCLA, Los Angeles, CA

Arginine biosynthesis in *Neurospora crassa* is regulated primarily by feedback inhibition of the first two enzymes of the pathway, N-acetylglutamate synthase (AGS) and N-acetylglutmate kinase (AGK). AGS and AGK are encoded by unlinked genes, *arg-14*and *arg-6*. Previous genetic studies suggested a coordinate mechanism of inhibition mediated by interaction between AGS and AGK: mutations in the gene for AGK (*arg-6*) affect not only the activity and feedback sensitivity of AGK, but also of 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. This domain has been termed the fungal domain because it is not present in bacterial counterparts of this enzyme. Deletion of the fungal domain results in a catalytically active AGK, yet renders AGS insensitive to feedback inhibition by arginine. This truncated AGK can also activate AGS when transformed into strains with an otherwise inactive AGS. This supports an important role for the fungal domain in sensing arginine for coordinated feedback inhibition.

43. Identification of cosmids that functionally complement deficiencies in polyketide pigment biosynthesis in the chestnut blight fungus. Tara M. Sirvent and Alice C.L. Churchill, Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY, USA.

Cryphonectria parasitica synthesizes a family of orange and yellow pigments, which are aromatic polyketides that exhibit numerous and diverse biological activities *in vitro*. These include antimicrobial and antiviral activities, cytotoxicity, apoptosis and cytochrome P450 induction, radical scavenging and active oxygen production, and tyrosine kinase inhibition. Our goal is to determine the role(s) of the polyketide pigments in the biology of *C. parasitica* by targeted disruption of the polyketide synthase (PKS) gene predicted to encode the first enzymatic step in the pigment biosynthetic pathway. We cloned ten unique PKS-like fragments from *C. parasitica* using PCR amplification with degenerate primers designed to conserved domains of known PKSs. These fragments were used as probes to a genomic cosmid library of a wild type, pigmented strain of the fungus. Forty-two unique cosmids hybridized to one or more PKS fragments; one fragment failed to hybridize to any members of the library. Cosmids were grouped according to their hybridization patterns to each PKS fragment, and four groups of five or six cosmids were transformed into a pigment- and sporulation-deficient ("white") *C. parasitica* mutant, which is also methionine-auxotrophic. Among many white hygromycin-resistant transformants, six independently isolated transformants produced orange pigment. Complementation occurred in the presence of transforming DNA from each of 3 independent cosmid groups. Orange-pigmented, monoconidial isolates were mitotically stable in the absence of hygromycin selection and exhibited increased sporulation in comparison with the white mutant. Efforts are underway to identify and sequence a single cosmid that confers orange pigmentation and sporulation to several independent pigment mutants of *C. parasitica*.

44. Characterization of the Arg-13 Mitochondrial Carrier Transport Protein. Rey Renato G. David and R. L. Weiss. UCLA, Los Angeles, California.

Metabolic processes take place in different compartments in eukaryotic cells. Intracellular compartments, such as the mitochondria, harbor enzymes and substrates that participate in specific metabolic pathways. Arginine biosynthesis in *Neurospora crassa* is an accessible model system to understand compartmentation. In *N. crassa*, glutamate is converted into citrulline inside the mitochondria, and citrulline is exported into the cytosol to be converted into arginine. We are investigating the role of the Arg-13 mitochondrial transport protein in the transport of arginine pathway metabolites across the mitochondrial membrane. The *arg-13* gene has been cloned and inserted into a pET3a vector. Arg-13 has been overexpressed in BL21-SI cells and purified. Characterization of Arg-13 involves determining the submitochondrial localization of the protein using polyclonal antibodies and using proteoliposome transport assays to determine substrate(s) specificity, transport activity and mode of transport. Preliminary results suggest that Arg-13 is an ornithine transporter. Characterization of Arg-13 will help elucidate the

role it plays in arginine metabolism and add another piece of information towards understanding the role of compartmentation in metabolic regulation.

45. Analysis of genes related with fumonisin production in *Gibberella fujikuroi*. González-Jaén, M.T, S. Mirete, E. Errasquin, B. Patiño, G. Mulè, C. Vázquez. Genetics, University Complutense of Madrid, Madrid, Spain

Toxin production is a complex process in which a number of genes are involved, such as biosynthetic genes or efflux pumps,and would be regulated by a net of interactions with other relevant processes in fungal development and stress responses. The reports on the organization of this genes in several cases reveals an impairment with the rest of nuclear genes in terms of similarity, for instance, and the possible involvement of horizontal transfer or transposon events. We have analyzed the occurrence and the expression of several genes included in the putative fumonisin biosynthetic cluster in species included in the *G. fujikuroi* species complex and particularly within *F. verticillioides* where two subpopulations could be differentiated. Those strains isolated from banana seemed to lack those fumonisin biosynthetic genes in the same way as other *G. fujikuroi* species, which generally are reported as fumonisin non producers (*F. sacchari*, *F. subglutinans*, *F. thapsinum* and *F. circinatum*). An analysis of efflux pump coding genes related with fumonisin production is also presented.

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Cell Biology

46. Msb3p and Msb4p, a pair of Rab GAPs, link actin organization to secretion in *S. cerevisiae*. Erfei Bi, Dept. of Cell & Dev. Biol., Univ. of Pennsylvania, Philadelphia, PA 19104-6058

Budding in *S. cerevisiae* occurs by polarized secretion to the daughter cell, which is directed by the polarized actin cytoskeleton. Cdc42p, a small Rho-type GTPase, plays an essential role in the polarization of the actin cytoskeleton, whereas Sec4p, a Rab GTPase, plays an essential role in the transport and tethering of the post-Golgi vesicles to the daughter cell. Previously, we have shown that Msb3p and Msb4p could function as dosage-dependent suppressors of *cdc42* mutants and displayed GTPase-activating protein (GAP) activities for a number of Rab GTPases *in vitro*, including Sec4p. Now, we provide evidence to indicate that Msb3p and Msb4p function as "arginine-finger" GAPs for Sec4p *in vivo* and the GAP activity of Msb3p and Msb4p is essential for their functions in secretion and actin organization. In addition, we have shown that Msb3p and Msb4p interact directly with Cdc42p and are in the same complex with Bni1p, Spa2p, and Bud6p, all of which are involved in the formation and/or attachment of actin cables to the presumptive bud site. Thus, Msb3p and Msb4p may coordinate actin organization to secretion by interacting with both the Cdc42p and the Sec4p GTPase modules.

47. The accumulation of cytoplasmic dynein and dynactin at microtubule plus-ends in Aspergillus nidulans is kinesin dependent. Jun Zhang*, Shihe Li*, Reinhard Fischer** and Xin Xiang* *Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, U. S. A **Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str. D-35043 Marburg, Germany

The mechanism(s) by which microtubule plus-end tracking proteins are targeted is unknown. In the filamentous fungus *Aspergillus nidulans*, both cytoplasmic dynein and NUDF, the homolog of the LIS1 protein, localize to microtubule plus ends as comet-like structures. Here we show that NUDM, the p150 subunit of dynactin, also forms dynamic comet-like structures at microtubule plus ends. By examining GFP-fusion proteins in different loss-of-function mutants, we demonstrate that dynactin and cytoplasmic dynein require each other for microtubule plus-end

accumulation, and the presence of cytoplasmic dynein is also important for NUDF's plus-end accumulation. Interestingly, deletion of NUDF increases the overall accumulation of dynein and dynactin at plus ends, suggesting that NUDF may facilitate minus-end directed dynein movement. Finally, we demonstrate that a conventional kinesin, KINA, is required for the microtubule plus end accumulation of cytoplasmic dynein and dynactin, but not of NUDF.

48. The mitogen-activated protein kinase Osc1 confers the response to high osmotic stress and fungicide sensitivity in *Colletotrichum lagenarium*. Kaihei Kojima, Yoshitaka Takano, Taisei Kikuchi, and Tetsuro Okuno. Department of Agriculture, Kyoto University, Kyoto, Japan

Colletotrichum lagenarium, the causal agent of cucumber anthracnose disease, invades the host plant using specialized infection structures called appressoria. We have shown two mitogen-activated protein kinase (MAPK) genes, *CMK1* and *MAF1*, are required for fungal infection process including appressorium formation in this fungus. Here, we identified*OSC1* encoding a MAPK of *C. lagenarium* that belongs to the *S. cerevisiae* Hog1 MAPK subfamily. *OSC1* encodes a 357 amino acid protein with 96% identity to the *Magnaporthe grisea* Osm1 MAPK and 80% identity to the *S. cerevisiae* Hog1 MAPK. Target disruption of *OSC1* had no detectable effect on mycelial growth, conidiation, and pathogenicity to cucumber plants. However, the *osc1*mutants exhibited reduced growth on high osmotic media compared with the wild-type strain, indicating that Osc1 is involved in response to high osmolarity like yeast Hog1. We also found that the *osc1* mutants were resistant to a phenylpyrrole fungicide fludioxonil, indicating that the Osc1 MAPK pathway confers sensitivity of the fungicide. When conidia of the wild-type strain were inoculated on cucumber leaves in the presence of fludioxonil, they failed to form lesions efficiently. In contrast, the *osc1* mutants exhibited normal pathogenicity even in the presence of fludioxonil, indicating that inhibition of fungal infection by fludioxonil depends on *OSC1*. These suggest that proper regulation of the Osc1 MAPK is required for fungal pathogenicity of *C. lagenarium*.

49. Altered ionic homeostasis and phenotypic suppression of the *Neurospora crassa* COT1 kinase mutant by environmental stresses. Oded Yarden and Rena Gorovits. Department of Plant Pathology and Microbiology and The Otto Warburg Center for Agricultural Biotechnology, 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 that is structurally related to the human myotonic dystrophy kinase, which when impaired confers a disease that involves changes in cytoarchitecture and ion homeostasis. When grown at restrictive conditions, *cot-1* cultures exhibited enhanced medium acidification rates, increased relative abundance of sodium and higher intracellular glycerol content, indicating an ionic homeostasis defect in the hyperbranching mutant. The presence of ion transport blockers, increased medium osmoticum (NaCl or sorbitol), H_2O_2 or ethanol, suppressed the *cot-1* phenotype to various degrees, suggesting that COT1 is linked with stress signaling. Environmental suppression of the *cot-1* phenotype was accompanied by a reduction in PKA activity. Direct inhibition of PKA with KT-5720 partially suppressed the *cot-1* phenotype, but in contrast to that observed with extragenic ropy suppressors of *cot-1*, did not change COT1 polypeptide expression patterns in the mutant. We suggest that COT1 function is linked to the PKA pathway, which is altered in a *cot-1* background and that reducing PKA activity bypasses the requirement for a fully functional COT1. This work was supported by the Israel Science Foundation.

50. Protein Kinase C interacts with and regulates the protein levels of the Neurospora photoreceptor WC-1. Franchi L., Fulci V., Macino G. Department of Biology, Universita' La Sapienza, Rome, Italy

The Neurospora photoreceptor WC-1 is transiently phosphorylated in response to light and this correlates with the transient increase of the light-regulated mRNAs levels, and with a progressive, light induced degradation of WC-1. Here we have investigated the function of PKC activity on the Neurospora photoreceptor WC-1, and on the regulation of light responses. We used PKC activators and inhibitors to test the effects on WC-1 phosphorylation and stability. We show that PKC activators keep WC-1 hyperphosphorylated even after several hours of constant light, inducing a degradation of the WC-1 protein. We show that PKC interacts with WC-1 in vivo and that endogenous PKC phosphorylates WC-1 on the zinc-finger region in vitro. To understand the physiological meaning of these observations, we studied the function of PKC using dominant negative and constitutively active mutants of

the kinase. We found that a constitutively active PKC induces a dramatic decrease in WC-1 protein levels, and a decrease in the light induced transcription of the *al-2* mRNA. We see clear opposite effects in the presence of the dominant negative PKC. Furthermore, we have seen that PKC kinase activity is transiently light induced and it has an important function in the light regulated inhibition of mycelial growth. Our data indicate that the protein levels and the activity of the Neurospora photoreceptor WC-1, and the light regulated mycelial growth are regulated by PKC, which is, therefore, a novel component of the light signal transduction pathway directly interacting with and phosphorylating WC-1.

51. *Aspergillus nidulans* MATE mobile elements: evidence of RIPping? John Clutterbuck. Institute of Biomedical and Life Sciences, Anderson College, University of Glasgow, Glasgow Scotland,

MATE elements were identified as a result of investigations into the AMA1 sequence which allows autonomous replication of plasmids in *A. nidulans* (Aleksenko & Clutterbuck 1996 Mol. Microbiol. 19: 565-574). Five MATE elements can be identified in the Glasgow strains of *A. nidulans*, while wild isolates contain variable numbers, inserted at different sites. MATE sequences obtained from the Cereon Microbial Sequence Database show elements of 6097 bp, flanked by 9 bp target site repeats. The most notable sequence features are numerous "Spe" motifs, [(RWCTAGWYNNN)2-4], scattered throughout, and potential stem-loop structures at each end of the elements. No ORFs with significant homology have been identified, and the mechanism of transposition and relationship to plasmid replication are both unknown. MATE1a and MATE1b are inserted back-to-back on chromosome IV, separated by 374 bp of unique sequence. Partial sequencing indicates that these two copies are identical, but solitary elements on other chromosomes differ from MATE1 by numerous transition mutations. Comparisons between these solo copies indicate greater divergence, suggesting independent origins from MATE1 or its precursor. The mutated copies show both T-C and G-A substitutions in patterns suggesting independent mutation of each strand. The doublet preference for mutation is CpG > CpA, with other doublets rarely mutated. It is suggested that mutation may have occurred by a RIP-like mechanism, from which MATE1 elements are protected by their putatively telomeric chromosomal location.

52. Characterization of the *Aspergillus nidulans* morphogenetic mutants *hypA1* and *hypA6*. Sha Yu and Susan Kaminskyj Department of Biology, University of Saskatchewan, Saskatoon, Canada.

Aspergillus nidulans has tubular hyphae with highly polarized tip cell growth, and quiescent basal cells separated from the tip by a septum. This morphogenetic pattern requires genes including *hypA* to promote growth of tip cells and suppress growth of basal cells. Fungal homologues of *hypA* include *Saccharomyces cerevisiae TRS120*, which is essential and is required for Golgi transit, a stage in secretion required for polarized growth.*A. nidulans hypA* is not essential, but a *hypA* knockout strain grows very poorly *hypA* has two temperature sensitive, nonlethal, recessive alleles, *hypA1* and*hypA6*, which produce similar restrictive phenotypes: wide slow growing cells with thick walls. The *hypA1* lesion is G329R; sequencing shows the *hypA6* lesion is K885F plus E932K, which is being confirmed by complementation. The effect of *hypA1* and *hypA6* on secretion polarity being studied using the *in vivo* dye, FM4-64, that tags endomembranes. As expected, wildtype hyphae and the permissive phenotype of *hypA1* and *hypA6* have well organized endomembrane systems culminating in a Spitzenkörper. In contrast, *hypA1* and *hypA6* restrictive phenotypes have diffuse endomembrane arrays that are somewhat more concentrated at their growing tips, consistent with their poorly polarized wall deposition pattern.

53. Regulation of septation in *Aspergillus nidulans*. Bo Liu, and Y.-R. Julie Lee. Plant Biology, UC-Davis, Davis, CA.

Septation initiation network (SIN) involving a MAP kinase-like kinase cascade plays a pivotal role in temporal regulation of septation in fungi. But it is unclear how the SIN network initiates septation. In the filamentous fungus *Aspergillus nidulans*, after conidial germination, the first septum is assembled at or near the basal end of the hypha after three rounds of mitosis. Among SIN molecules, the novel evolutionarily conserved MOB1 protein associates with the last kinase in this kinase cascade. We have cloned cDNA of the *AnMOB1* gene in *A. nidulans*. By using a functional GFP-AnMOB1 fusion, the AnMOB1 protein was detected at the spindle pole body in interphase. In M phase, however, GFP-AnMOB1 appeared not only at the spindle pole body, but also along the central spindle and later at the septation site. Down-regulation of *AnMOB1* expression or deletion of *AnMOB1* allowed hyphal growth, but abolished septation and conidiation. We are now exploring whether over-expression of AnMOB1 or other SIN

molecules allows coupling of septation and mitosis after conidial germination in this filamentous fungus. Interestingly, AnMOB1 localization to the spindle pole body and the septation site was dependent on intact microtubules. Previously, we have shown that the cytoplasmic dynein is required for correct positioning of the septum, implying that this microtubule motor may play a role in the localization of SIN molecules. Therefore, we are testing whether the localization of AnMOB1 and other SIN proteins is dependent on microtubule-based motor proteins including dynein.

54. Is a 1,3 - beta - glucan synthase homolog an essential gene in *Coccidioides posadasii*? Ellen M. Kellner[1], Kris I Orsborn[1], Erin M Siegel[1], Marc J Orbach[2] and John N Galgiani[1]. [1]The Valley Fever Center for Excellence, Southern Arizona VA Health Care System, Tucson, AZ. [2]Department of Plant Pathology, University of Arizona, Tucson, AZ.

Beta glucans are major components of the cell walls of many fungi including Coccidioides spp. Because of this, they are targets for antifungal drugs and the 1,3 beta - glucan synthase inhibitor, caspofungin, has shown potential therapeutic benefit for treatment of coccidioidomycosis. In *Paracoccidioides brasiliensis*, *Aspergillus nidulans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*, the FKS subunit of 1,3 beta - glucan synthase which is the target of caspofungin, appears to exist as an essential single copy gene. We identified, cloned and sequenced the gene, GS1, from the Silveira strain of *Coccidioides posadasii* which showed a high degree of similarity to FKS genes in other species. In order to validate GS1 as a target for antifungal therapy in Coccidioides spp., we sought to create a null allele through homologous gene replacement. We replaced the GS1 coding sequences of *C. posadasii* with a dominant selectable marker for hygromycin resistance using the *Agrobacterium tumifaciens* T-DNA transfer technique. PCR and Southern blot analyses showed all 24 hygromycin resistant transformants harbored a wild-type allele of GS1. However, 6 (25%) also contained a homologous gene replacement and therefore were likely heterokaryons. Heterokaryotic transformants that included homologous GS1 replacements were defective in arthroconidiation as compared to strains with ectopic construct integrations or wild-type strains. Homologous and ectopic transformants were purified through streaking for single colonies and subsequent purification of arthroconidia from isolated colonies. All purified strains maintained a wild-type allele of GS1. These findings support an essential role for GS1 in Coccidioides spp. an attractive target for further antifungal drug discovery.

55. The phenotype of the *Neurospora crassa cot-5* mutant, defective in a mannosyltransferase, can be suppressed by increased medium osmoticum. Zipora Resheat-Eini, Rena Gorovits, Oded Yarden. Department of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Science, The Hebrew University of Jerusalem, Rehovot 76100, Israel

Neurospora crassa colonial temperature sensitive mutants, (*cot-1, cot-2, cot-3, cot-4* and *cot-5*) form compact, highly branched colonies. As the defects in these mutants also involve apparent alterations in cell wall morphology, we examined the effect of changes in environmental osmoticum on the mutants. When grown in the presence of 0.75-1M sorbitol the hyper branching phenotypes of *cot-1, cot-4* and *cot-5* were suppressed to various degrees. *cot-5* exhibits an altered COT1 polypeptide expression pattern, which suggests that COT5 and COT1 may be functionally linked. We have cloned *cot-5*, by complementation, and have determined it encodes a mannosyltransferase which is highly similar to the *Saccharomyces cerevisiae* dolichol pathway ALG2 protein. The mutation in *cot-5* consists of a single base substitution leading to the formation of an amber termination codon at amino acid 37. Treatment with Tunicamycin (a specific inhibitor of *N*-glycosylation) inhibits hyphal elongation and induces hyperbranching in a manner that mimics the *cot-5* phenotype. Even though COT1 has several potential *N*-glycosylation sites, we have not found evidence for altered COT1 glycosylation in a *cot-5* background.

56. Isolation and characterization of genes encoding dynein heavy chain, *dhc1* and*dhc2*, and Ras GTPase-activating protein, *gap1*, from *Schizophyllum commune*. D. Schubert and E. Kothe, FSU, Jena, Germany.

In the homobasidiomycete *Schizophyllum commune* mating is in part controlled by a pheromone receptor system. Signal transduction starting from an activated pheromone receptor results in fast nuclear migration and is a prerequisite for establishment of the fertile dikaryon. We are studying the roles of cytoplasmic dynein and Ras GTPase-activating protein in these processes. The heavy chain of cytoplasmic dynein is encoded by two separate genes in *S. commune, dhc1* and *dhc2*. Unlike *dyn1* of *Ustilago maydis*, *dhc1* comprises only the N-terminal dimerization domain of the protein. ATP-binding sites and microtubule-binding domain are encoded by the second

gene, *dhc2*. Deletion of *dhc2* led to viable monokaryotic strains. They showed slow growth and a tendency of hyphal knot formation resulting in colonies having a complex morphology. In matings with wildtype strains delta*dhc2* mutants were able to accept nuclei, but dikaryotization was slower than in the wildtype. The Ras GTPase-activating protein gene *gap1* showed the highest identity to*gap1* from *Schizosaccharomyces pombe. gap1* deletion mutants were able to mate both with wildtype and delta *gap1* strains. Dikaryons heterozygous for delta *gap1*developed normally. However, homozygous delta *gap1* dikaryons showed abnormal clamp cell formation which affected the backward bending of clamp cells. In addition, development of lateral branches from clamp cells could be observed.

57. A homologue of Ste6p, the a-factor transporter in *Saccharomyces cerevisiae*, functions bilaterally in *Cryptococcus neoformans*. Yen-Ping Hsueh and Wei-Chiang Shen. Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan

Fungal pheromones have been demonstrated to function in the initial recognition of the fungal mating process. One type of peptide pheromones, identified in the Ascomycetes and Basidiomycetes, terminate in a conserved CAAX motif which triggers sequential post-translational modifications of the pheromone precursors. Among this type of peptide pheromones, a well-studied one is the **a**-factor of *Saccharomyces cerevisiae*. The mature **a**-factor is exported from the cell via an alternative mechanism involving the ATP-binding cassette transporter Ste6p, which is distinct from the typical secretory pathway utilized by most peptides. Unlike the Ascomycetes, the basidiomyceteous fungi produce only CAAX motif containing lipopeptide pheromones. *Cryptococcus neoformans*, a human pathogenic basidiomycetous yeast, causes the life-threatening meningoencephalitis mainly in individuals with compromised immune functions. Virulence studies of the congenic pair of *C. neoformans* strains have shown that *MATalpha* cells are more virulent than*MAT**a*** cells. Characterization of mating pheromone genes in the *MATalpha* strains have suggested an autocrine signaling loop may function and contribute to the virulence of the *MATalpha* cells. To further address the role of pheromone in the signaling loop, we have identified *STE6* homologue in the *C. neoformans* genome project at SGTC and begun to dissect its function. By disrupting the*STE6*, we found that *ste6* mutants in either *MATalpha* or*MAT**a*** background showed partially impaired mating function, although slight differences were noticed. However, when *ste6 MATalpha* and*MAT**a*** mutants cross with each other, the mating process was nearly completely abolished. Our data indicates that the STE6 functions bilaterally and is required but not essential for mating in *C. neoformans*.

58. Expression profiling of the ectomycorrhizal interaction between birch and different compatible strains of *Paxillus involutus*. Andres Schützendübel[1], Antoine Le Quere[1], Thomas Johannson[1], Susanne Erland[1],Anders Tunlid[1] [1]Department of Microbiol Ecology , University of Lund, Sweden

A lot of investigations led to the assumption that a continuum between parasitic, saprophytic and mutualistic living basidiomyctes exists and the Ectomycorrhizal-(ECM) fungi have evolved from saprophytic precursors, but there are also multiple reversals to a free living lifestyle (Hibett et al., 2000).Differences in the form of interaction exist not only between different species and groups also between different strains of the same species mutualistic as well as saprophytic lifestyles and intraspecific strong host specificity has been observed (Brundett, 2002). The aim of this study is to investigate the different forms and changes of interactions between roots and ECM-fungi. Birch and *Paxillus involutus* were chosen as a model system. We screened several strains of *P. involutus* on its ability to develop a functioning mycorrhizal interaction with birch. Two strains, isolated from different hosts showed strong differences in establishing mycorrhizal structures. In the non-mycorrhizal strain no Hartig-net was formed and no inhibition of lateral root growth during the early phase of the interaction (one day – seven days) was observed. Additional differences in physiological interaction were detected. By microarray analysis we are now investigating differences in gene expression between the two strains during mycorrhiza development.

Literature cited: Hibett et al., (2000) Nature 407: 506-508 Brundett, (2002)New Phytol 154: 275-304

59. Checkpoint control genes in *Neurospora crassa*. Chizu Ishii and Hirokazu Inoue, Laboratory of Genetics, Saitama University, Saitama, Japan

We have searched for genes related to cell cycle functions on the Neurospora genome database. Among 28 ORFs that showed homology in blast search, five candidates were located close to known genes. By transformation using

genome fragments, we confirmed that*un-14*, *mus-9* and *mus-21* were homologs respectively of *CDC25*,*MEC1*, and *TEL1* of *Saccharomyces serevisiae*. As a *tel1* mutant in yeast has no apparent phenotype as a DNA repair mutant, we characterized the *mus-21*mutant in particular. The *mus-21* mutant was highly sensitive to methyl methanesulfonate, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, *tert*-butyl hydroperoxide and slightly sensitive to UV, camptothecin, hydroxy urea and hydrogen peroxide. The *mus-21* mutant was sterile when crossed homozygously. Cytological investigation revealed that the defect(s) was at meiosis I. The *mus-21* mutation induced synthetic lethality in combination with the *mus-9* mutation. Epistatic grouping of the *mus-21* mutation based on mutagen sensitivity of double mutants showed that this mutation was involved in the *uvs-6* (*RAD50* homolog) group, but synergistic to one member of this group, the *mei-3* (*RAD51* homolog) mutation. These results indicate that Neurospora uses two redundant but essential signaling pathways for checkpoint control and that, as in yeast, recombination repair has two independent pathways with the Neurospora Tel1 homolog plays a role in the Rad51 independent pathway.

60. A class V myosin involved in chitin synthase delivery towards growth sites of*Ustilago maydis.* Isabella Weber, Gagan Gupta and Gero Steinberg. Max-Planck-Institute for terrestrial Microbiology, Karl-von-Frisch Strasse D-35043 Marburg, Germany.

Class V myosin motors utilize F-actin to support intracellular traffic in eukaryotic cells. Our knowledge about their existence and function in fungi is restricted to the yeasts *S. cerevisiae* and *S. pombe*. In the former, a class V myosin is thought to support polar growth by delivering chitin synthase towards the growth region. We identified a class V myosin in *U. maydis* (Myo5) that is involved in polar growth and pathogenicity (see poster by Gupta et al.). We checked for a role of Myo5 in polar depositioning of chitin and localization of chitin synthase by wheat-germ agglutinin staining and a cross reactive antibody against Chs2p from *S. cerevisiae* (Sietsma et al. 1996). Both chitin and chitin synthase were found to form a gradient towards the growing bud of wild-type cells. This localization was insensitive to microtubule disruption by Benomyl, but both the actin inhibitor Latrunculin and the myosin drug BDM severely affected chitin and chitin synthase distribution. This suggests that the actomyosin system supports directed transport of chitin synthases towards the growth region. A temperature sensitive Myo5 mutant was normal at 20°C but showed defects in morphogenesis at 28°C, a temperature that did not affect the actin cytoskeleton. Interestingly, polar distribution of chitin and chitin synthase was disrupted in $Myo5^{ts}$ cells upon temperature shift, but chitin and chitin synthase became repolarized after 3h. This redistribution of chitin and chitin synthase in $Myo5^{ts}$ cells was also sensitive to Latrunculin and BDM, indicating that after 2-3 hours an actin-based transport mechanism compensates for the inactivation of the temperature sensitive allele of *myo5* . This suggests that, in contrast to the yeast *S. cerevisiae*, other myosins participate in polar growth and chitin synthase distribution in *Ustilago maydis*. Recent progress in elucidating the mechanisms underlying this phenomenon will be presented.

61. Cloning and characterization of a two-component histidine kinase gene of *Cochliobolus heterostrophus* involved in osmotic adaptation and dicarboximide resistance. Akira Yoshimi, Chihiro Tanaka and Mitsuya Tsuda. Laboratory of Environmental Mycoscience, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan.

A two-component histidine kinase gene was cloned and sequenced from a plant pathogenic fungus *Cochliobolus heterostrophus*. The predicted protein possessed the conserved histidine kinase domain, the response regulator domain and the six-tandem repeats of 92 amino acids in the N-terminus. The deficiency of *C. heterostrophus Dic1* mutant was complemented with introduction of the wild type histidine kinase gene, suggesting the *Dic1* gene encoded the putative histidine kinase. All the *Dic1* mutant alleles exhibit osmotic sensitivity and resistance to dicarboximide and phenylpyrrole fungicides, and they can be classified into three types on the basis of their phenotypes. To elucidate molecular basis of their allelic nature, we sequenced the genes from ten *Dic1* mutants. The null mutants of Dic1p and the mutants with deletion or point mutation in the N-terminal repeat region were highly sensitive to osmotic stress and highly resistant to both fungicides. The single amino acid change within the kinase domain or the regulator domain conferred various sensitivity to osmotic stress and moderate resistance to the fungicides. These results suggest that this predicted protein, especially the amino acid repeat region, has an important function in the osmotic adaptation and the fungicide resistance. Further functional analysis of the histidine kinase based on site-directed mutagenesis is in progress.

62. Characterization of the mechanism of action of PAF, an antifungal protein secreted by *Penicillium chrysogenum*. Florentine Marx[1], Christoph Oberparleiter[1], Wolfgang Burgstaller[2], Renate Weiler-Görz[1] and Lydia Kaiserer[1]. [1]Department of Molecular Biology, Innsbruck, Austria.[2]Intstitute of Microbiology, Innsbruck, Austria

The filamentous fungus *Penicillium chrysogenum* secretes the antifungal protein PAF. So far, no information is available on the exact mode of action of this small, highly basic and cysteine rich protein. We characterized the protein function in more detail and show that secreted PAF retains its activity over a broad pH range and resists to high temperature and protease digestion. The protein is effective against the growth of a variety of phytopathogenic and opportunistic human pathogenic molds. PAF reduces spore germination and hyphal extension in a dose dependent manner and induces stress responses, including morphological changes, plasma membrane leakage, generation of radicals and metabolic inactivity. These detrimental effects cannot be antagonized by cations and can be neutralized only to some extent by hypertonic growth conditions, suggesting that the cell wall or membrane are not the primary target sites of PAF. This finding is supported by the intracellular localization of PAF in sensitive fungi. Our results demonstrate that the mechanism of action of PAF differs from that of other known antifungal proteins. Thus PAF represents a new antifungal agent potent to inhibit the growth of harmful molds.

63. The WASP-homolog Wal1p is required for morphogenesis, hyphal growth and full virulence of the human fungal pathogen *Candida albicans*. Andrea Walther, Nir Osherov and Jürgen Wendland Dept. of Microbiology, Friedrich-Schiller Univ. and Hans-Knoell-Institute for Natural Products Research, Jena, Germany;Dept. of Human Microbiology; Sackler School of Medicine; Tel-Aviv Univ., Tel Aviv, Israel.

The dynamic reorganization of the actin cytoskeleton underlies processes of cell polarization, morphogenesis and cytokinesis. Cell proliferation and morphogenesis requires two distinct steps (i) polarity establishment to define new areas of growth eg for bud formation and (ii) maintenance of polarized growth eg to form hyphae. Previously we characterized the essential role of Rho-type GTPases in these processes in the filamentous fungus *Ashyba gossypii*. Here we report the identification and characterization of a gene required for the maintenance of polarized growth in *Candida albicans*. This gene, encoded by CaWAL1, belongs to the family of Wiskott-Aldrich syndrome-Like proteins that are known for their role in actin filament nucleation via activation of the Arp2/3 complex. Homozygous *C. albicans* wal1/wal1 cells diplay several mutant phenotypes that are all based on defects in cortical actin cytosekeleton assembly. (i) Mutant cells display a random budding phenotype instead of the wildtype bipolar budding pattern. (ii) Mutant cells exhibit a cytokinesis defect. (iii) Yeast cells appear round in shape instead of ellipsoidal as wildtype (or heterozygous WAL1/wal1 cells) indicating loss of poalrized secretion. (iv) hyphal induction of wal1/wal1 cells using either spider medium or serum both in liquid medium or on plates only results in the formation of pseudohyphae with a drastic decrease in filament formation. (v) And, finally, mutant cells display a decreased virulence (about 1% of wildtype level) in a mouse model. Our results demonstrate that cortical actin cytoskeletal organization and polarized secretion are crucial for morphogenesis, hyphal formation and virulence in *C. albicans*.

64. Maintenance of polarized hyphal growth requires a WASP family member protein in the filamentous fungus *Ashbya gossypii*. Andrea Walther and Jürgen Wendland. Dept. of Microbiology, Friedrich-Schiller University and Hans-Knoell-Institute for Natural Products Research, Jena, Germany

Mycelium formation in filamentous fungi is based on a two step process requiring the actin cytoskeleton for both stages. First, the polarity establishment machinery is involved in chosing a site at which a new hyphal tip is formed. Second, maintenance of polarized cell growth keeps secretion of cell wall compounds polarized to the hyphal tip and results -in contrast to yeast cells- in hyphal elongation. Previously, we identified two genes required for the first step, i.e. polarity establishment, in Ashbya gossypii, encoded by AgCDC42 and AgCDC24. Here we report the identification and characterization of a gene required for the maintenance of hyphal growth. This gene, AgWAL1, encodes a protein with similarity to the Wiscott-Aldrich Syndrome protein family. WASP family members regulate the organization of the cortical actin cytoskeleton via interaction with the Arp2/3 complex and activation of actin filament nucleation. Agwal1 mutant spores germinate unilaterally and do not display the bipolar branching pattern of wildtype spores. Growing hyphal tips exhibit severe defects in hyphal elongation after tip formation. This results in swollen hyphae that reveal extended periods of isotropic growth as determined by time lapse microscopy. Our results indicate that an AgWal1p-independent mechanism ensures the reorganization of the actin cytoskeleton during

hyphal tip formation whereas polarized organization of actin cortical patches and the maintenance of hyphal growth is dependent on AgWal1p.

65. Characterization of a secretion-related small GTPase encoding gene in *Aspergillus niger*. A.F.J. Ram[1,2], X.O. Weenink[1], P.J. Punt[2] and C.A.M.J.J. van den Hondel[1,2]. [1)]Leiden University, IMP, Wassenaarseweg 64, 2333 AL, Leiden, The Netherlands, [2)] TNO-Nutrition, AMGT, 3700 AJ Zeist, The Netherlands.

Filamentous fungi, such as *Aspergillus niger*, have the capacity to secrete large amounts of enzymes into their environment. Transport of these proteins through the secretion pathway is mediated by vesicles and controlled by highly conserved secretion related small GTPases of the Rab/Ypt family. Based on this conservation, we have cloned seven secretion related small GTPases (srg genes) from *A. niger* (Punt, et al., 2001). For the *A. niger srgC* gene, a putative homologue of the *S. cerevisiae ypt6* gene, a disruption mutant was generated. The *srgC* disruption mutant is viable but grows slower and more compact at 30°C compared to the wild-type strain. At higher temperatures this phenotype becomes more severe and results in a conidiation defect. In order to monitor protein secretion and protein transport to the vacuole, we have made GFP-based secretion reporter proteins. In both the wild type strain and in the *srgC* deletion strain, the CPY-GFP fusion protein is efficiently targeted to the vacuole. However, the disruption of the *srgC* gene resulted in a fragmentation of vacuoles, indicating a function of SrgC at a later step than the Golgi to vacuole transport step, e.g. a function in vacuolar fusion. Microscopical analysis of the *srgC*disruption mutant using the Glucoamylase-GFP fusion protein showed partial intracellular accumulation of the GFP fusion protein indicating that the disruption of *srgC* also affects protein secretion.

66. Molecular characterization of the *ramosa-1* mutant in *Aspergillus niger*. A.F.J. Ram[1,2], M. Arentshorst[1], C. G. Reynaga-Pena[3], S. Bartnicki-Garcia[4], C.A.M.J.J. van den Hondel[1,2]. [1)]Leiden University, IMP, Leiden, The Netherlands,[2)]TNO-Nutrition AMGT, Zeist, The Netherlands, [3]Universidad de Guanajuato, Mexico, [4]Centro de Investigacion Cientifica y de Educacion Superior de Ensenada, Mexico.

Polarized growth of a leading hyphe of many filamentous fungi is characterized by the presence of a Spitzenkörper or Vesicle Supplying Centre (VSC). Molecular mechanisms underlying the establishment and maintenance of the Spk are unknown. To identify proteins involved in this process we have characterized the previously isolated apical branching mutant, *ramosa-1* (1). We have cloned the *ramosa-1* gene by complementation of the temperature sensitive phenotype. Homology searches indicate that the protein belongs to an evolutionary conserved family of proteins. For the *S. pombe* homolog, it has been shown that the protein interacts with a stress activated MAP kinase. Overexpression of the *ramosa-1* cDNA could rescue the *S. cerevisiae* gene deletion mutant, indicating a functional conservation between the two homologs. 1) C.G. Reynaga-Pena and S. Bartnicki-Garcia. 1997. Apical branching in a temperature sensitive mutant of *Aspergillus niger*. Fungal Genetics and Biology 22, 153-167.

67. Identification and role of peroxisomal ABC transporters in the sexual development of the filamentous fungus *Podospora anserina*. S. Boisnard, D. Zickler, M. Picard and V. Berteaux-Lecellier. Institut de Génétique et Microbiologie, UMR 8621 Université Paris-Sud Bat. 400, 91405 Orsay cedex France.

Peroxisomes play essential roles in cellular metabolism, their deficiencies are responsible for severe (and often lethal) human disorders. In the filamentous fungus *Podospora anserina*, we have previously observed that the peroxisome assembly *pex2* mutants (Zellweger Syndrome in humans) are impaired in sexual differentiation (Berteaux-Lecellier et al., 1995). Moreover, we have shown that expression of the human ABC transporter PMP70 in a *P. anserina pex2* mutant strain partially restores both peroxisome biogenesis and sexual differentiation (Boisnard et al., submitted). This result ascertains a role for peroxisomes in sexual development in this fungus. Interestingly, our study has also disclosed an unexpected detrimental defect of h *PMP70*cDNA expression in a wild-type background (*pex2+*): it leads to an abnormal distribution of both nuclei and peroxisomes during sexual differentiation. This last discovery prompted us to clone and sequence the two "expected" genes encoding peroxisomal ABC transporters in *P. anserina*. The experiments performed with the first peroxisomal ABC transporter pABC1, which display 35% identity with PMP70, confirm the results obtained with the human transporter. Further studies are in progress with the second gene *pABC2* and should help to understand the involvement of peroxisomal ABC transporters in differentiation as well as their physiological role(s).

68. The CBEL glycoprotein of *Phytophthora parasitica* var. *nicotianae* is involved in cell wall deposition and cellulose sensing. E. Gaulin[1,2], A. Jauneau[1], F. Villalba[3], Mt. Esquerre-Tugaye[1], M. Rickauer[1] and A. Bottin[1]. [1] Pôle de Biotechnologie Végétale,31326 Castanet-Tolosan, FRANCE. [2] Dept Plant Pathology, OARDC, 1680 Madison Avenue, Wooster, OH 44691-44096, USA. [3]Bayer Cropscience,69263, Lyon, FRANCE

Phytophthora parasitica var. *nicotianae*, an oomycete pathogen of tobacco, produces a Cellulose-Binding Elicitor Lectin (CBEL) which is cell wall-localized and induces defense in plants. The biological role of CBEL in *Phytophthora* was investigated by examining the phenotype of strains where *CBEL* expression had been silenced by transformation. When grown on cellophane membranes, the silenced strains were strongly reduced in substrate adhesion, whereas the *CBEL*-expressing strains adhered strongly to the membrane, in contact with which they formed lobed hyphal structures. Electron microscopy observations showed abnormal paramural appositions in hyphae of the silenced strains ; furthermore these strains did not branch in contact to flax cellulose fibers, in contrast to *CBEL*-expressing strains. The data indicate that CBEL is involved in cell wall apposition in *Phytophthora*, in adhesion to cellulose and in differenciation of specialized structures in response to this polysaccharide (1). 1- Gaulin, E. *et al.* (2002). *J Cell Science*, **115**, 4565-4575

69. Identification of photoreceptors that sense light and inhibit differentiation of*Cryptococcus neoformans*. Alexander Idnurm and Joseph Heitman. Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710.

Cryptococcus neoformans is a heterothallic basidiomycete yeast that infects animals, including humans. The infective particle of the fungus is hypothesized to be the basidiospore or spore resulting from haploid (monokaryotic) fruiting. Both mating and haploid fruiting are inhibited by light. This work aims at identifying the photoreceptor(s) involved in inhibition. Three genes (encoding a putative opsin, white collar 1 and phytochrome-like protein) have been identified as single copy genes in a serotype D *MAT* alpha strain of *C. neoformans*. The three genes have been mutated by targeted disruption, and mutants in the*MAT***a** mating type generated by crossing. Strains with two or three of the genes mutated are being constructed. The ability of these strains to mate and haploid fruit in the light compared to the dark, and the wavelength of inhibition, will be examined. Transcription of genes in the mating type locus and others identified through microarray analysis will also be examined in the wild-type and mutant strains grown in the light and dark.

70. The *apsA* and *apsB* genes of *Aspergillus nidulans* and cytoplasmic dynein. Vladimir Efimov. Department of Pharmacology, UMDNJ-RWJMS. Piscataway, NJ.

The *apsA* and *apsB* (anucleate primarysterigmata) mutants of *A. nidulans* have nuclear distribution defects that resemble those in the cytoplasmic dynein mutants, but are less severe. The *S. cerevisiae* homolog of *apsA*, *NUM1*, acts in the dynein pathway. The *apsA* gene interacts genetically with the *nudF* (*PAC1*, *LIS1*) gene of the dynein pathway (V.P. Efimov, Mol. Biol. Cell, 2003). Here the relation between the *apsA* and*apsB* genes and dynein is investigated by analysis of double mutants. Mutations in the *apsA* gene had no effect on the growth of dynein deletion mutants. In contrast, mutations in the *apsB* gene suppressed dynein deletion mutants and improved nuclear migration in their germlings. The suppression may stem from a defect in nuclear fixation that increases nuclear traffic in the *apsB* mutants (R. Suelmann et al., Mol. Microbiol., 1998). The results indicate that APSB regulates nuclear distribution independently of APSA and cytoplasmic dynein.
Financial Support: Scientist Development Grant from the American Heart Association.

71. Expression of a GFP-tagged histone H1 gene during ascus development in *Neurospora crassa*. N.B. Raju[1], M. Freitag[2] and R.L. Metzenberg[1,3].[1]Stanford Univ, [2]Univ Oregon, [3] UCLA.

A GFP-tagged histone H1 gene (*hH1-GFP*) under the control of the *ccg-1* promoter was inserted at the *his-3* locus (*his-3*$^{+}$*::hH1*$^{+}$*-eGFP*$^{+}$), hereafter called *H1-GFP*. We have examined the expression of *H1-GFP* in developing asci of homozygous and heterozygous crosses. In homozygous crosses (*H1-GFP* x *H1-GFP*), nuclei fluoresce brightly from karyogamy until the production of mature, multinucleate ascospores. In heterozygous crosses (*H1-GFP* x wild type), expression of *H1-GFP* is completely silenced, at least until after ascospores are delimited. Silencing does not extend into the autonomously developing ascospores. Nuclei in four of the eight spores begin to fluoresce 18 to 24 h

after ascospore delimitation. Fluorescence intensity increases for an additional 24 h as the ascospores form striations and begin to pigment. Additional mitoses occur in mature, black ascospores, and more than 60 nuclei can readily be seen in each of the four *H1-GFP* ascospores. The four ascospores without *H1-GFP* do not show fluorescence. *Sad-1*, which suppresses meiotic silencing of ectopically inserted genes (Shiu *et al*. 2001, Cell 107:905-916), also suppresses the silencing of *H1-GFP* in heterozygous asci. In *H1-GFP* x *Sad-1* crosses, expression resembles that of a homozygous *H1-GFP* cross: Nuclei fluoresce until after ascospores are delimited. The residual *H1-GFP* in non-*H1-GFP* nuclei gradually fades while nuclei in the four ascospores that contain *H1-GFP* continue to fluoresce brightly. These observations provide a clear visual demonstration of meiotic silencing by unpaired DNA. (Support: NSF grant 9728675 to D. Perkins, NIH grant GM35690 to E. Selker and NIH grant GM08995 to RLM.)

72. Characterization of Putative Calcium Transport Proteins in the Vacuole of *Neurospora crassa*. Stephen Abreu, Emilio Margolles-Clark and Barry Bowman. University of California, Santa Cruz CA 95064

The vacuole stores high concentrations of calcium and may play a central role in regulating calcium levels within fungal cells. We are investigating the role of three proteins, CAX, NCA-2 and NCA-3, that are hypothesized to transport calcium across the vacuolar membrane. The genes encoding these proteins were identified by similarity to calcium transport proteins in other organisms. Analysis of the genes indicates that CAX encodes a 12-helix protein that facilitates the exchange of calcium for protons. NCA-2 and NCA-3 both appear to be homologs of the PMC1 gene of *S. cerevisiae*. These genes appear to encode P-type ATPases that pump calcium into the vacuole. It is not known why *N. crassa* has two of these genes while*S. cerevisiae* has only one. To investigate the function of these genes we used the RIPing procedure to generate mutant strains. Inactivation of the *cax* gene produces no obvious growth phenotype, but the mutants are deficient in calcium transport into the vacuole when assayed *in vitro*. Mutant strains that lack the *nca-3* gene also grow like the wild type. However, inactivation of the*nca-2* gene causes slow growth and abnormal morphology. The *cax nca-2* double mutant is even more impaired. Other multiple mutants are being generated. A preliminary interpretation of these results is that the *cax* and *nca-2* gene products have partially redundant functions in the vacuole. The role of the *nca-3* gene is unknown.

73. NPKA is a novel putative p34^{cdc2}-related PITSLRE gene from *Aspergillus nidulans*, associated with the cellular DNA damage response. Gustavo H. Goldman, Marcela Savoldi, Maria Angela Castro Dani, Marcelo Afonso Vallim, Roy E. Larson[1], Maria Helena S. Goldman[2] FCFRP, [1]FMRP, and [2]FFCLRP, Universidade de São Paulo, Brazil

The DNA damage response is a protective mechanism that ensures the maintenance of genomic integrity during cellular reproduction. We have been using *A. nidulans* as a model system to genetically characterize the DNA damage response caused by the anti-topoisomerase I drug, camptothecin. Here, we report the molecular characterization of a novel p34*cdc2*-related PITSLRE gene, *npkA*, from *A. nidulans*. The PITSLRE kinases are a superfamily of protein kinases related to p34^{cdc2} that have been implicated in apoptosis. The*npkA* gene is transcriptionally induced by campthotecin and other DNA-damage agents, and its induction in the presence of camptothecin is dependent on the *uvsB*^{ATR} gene. Down-regulation of the *npkA* caused an increased induction of the *uvsC*^{RAD51}, *scaA*^{NBS1}, and *Anmre11* genes when *A. nidulans* is grown in the presence of camptothecin, suggesting that gene expression induced by DNA damage is dependent on the *npkA* gene. Furthermore, NPKA levels are apparently controlled by proteolysis when *A. nidulans* is grown in the presence of MMS. Our results suggest that the *npkA* gene may play a role in the DNA-damage signal transduction pathway in *A. nidulans*. Financial support: CNPq and FAPESP, Brazil

74. Different roles of the *mre11* complex in the DNA damage response in *Aspergillus nidulans*. Gustavo H. Goldman, Camile P. Semighini, Márcia R. V.Z. Kress Fagundes, Joseane C. Ferreira, Renata C. Pascon, Maria Helena S. Goldman[1]FCFRP and [1]FFCLRP, Universidade de São Paulo, São Paulo, Brazil

The hMRE11-hRAD50-NBS1 protein complex has emerged as a central player in the cellular DNA damage response. Mutations in *scaA*^{NBS1}, which encodes the apparent homolog of human nibrin in *A. nidulans*, inhibit growth in the presence of anti-topoisomerase I drug camptothecin. We have used the *scaA*^{NBS1}cDNA as a bait in a yeast two-hybrid screening and report the identification of the *A. nidulans Anmre11* homologue. The inactivated *Anmre11* strain (TMRE11) was more sensitive to several DNA damaging and oxidative stressing agents. Septation in *A. nidulans* is dependent not only on the *uvsB*^{ATR} gene but also on the *mre11* complex. *scaA*^{NBS1} and *Anmre11*

genes are both involved in DNA replication checkpoint while *Anmre11* gene is involved in the intra-S-phase checkpoint. SCAA^{NBS1} and ANMRE11 also participate in G2-M checkpoint upon DNA damage caused by 4-NQO. Homologous recombination is affected by the *scaA1* mutation since this mutant does not have the ability of integrating exogenous DNA introduced by DNA-mediated transformation in a homologous way. The *scaA*^{NBS1} gene is important for ascospore viability while *Anmre11* gene is essential for the sexual fertility in *A. nidulans*. In addition, the *mre11* complex and *uvsC*^{RAD51} genes are highly expressed at mRNA level during the sexual development. Financial support: CNPq and FAPESP, Brazil

75. The new subfamily of CuZn Superoxide Dismutase is involved in Branching and Hyphal Growth in *Candida albicans*. Mikhail Martchenko, Doreen Harcus, and Malcolm Whiteway. Biotechnology Research Institute (NRC), Montreal, Canada.

Candida albicans is an opportunistic human pathogen that can cause life-threatening systemic infections in immunocompromised. It is possible that in order to protect against damaging superoxide radicals produced in the phagosome *C. albicans* express different classes of superoxide dismutases (SOD). Superoxide dismutase converts superoxide radicals into less damaging H_2O_2. *C. albicans* is known to express Cu/ZnSOD (*SOD1*) and MnSOD (*SOD3*) in the cytosol and MnSOD (*SOD2*) in the mitochondria. Through the use of cDNA Microarray technology and the sequence of *C. albicans* genome we report the existence of three additional Cu/Zn superoxide dismutases, *CaSOD4*, *CaSOD5* and *CaSOD6*. The transcription of *CaSOD5* is upregulated in yeast to hyphal transition of *C. albicans*, which is thought to be involved in the pathogenesis of this organism.*CaSOD5* is highly expressed when *C. albicans* cells are challenged with osmotic or with oxidative stresses at 30C but not at 37C. *Efg1* and *Rim101* transcription factors are involved in *CaSOD5* regulation. Deletion of *CaSOD5* produced a viable mutant strain, which showed the same resistance to macrophage attack as its parental strain. *CaSOD5* mutant strain showed a temperature sensitive lysine auxotrophy, and*CaSOD5* was found to be involved in hyphal branching and in hyphal growth. This work defines a new subfamily of CuZn superoxide dismutases, and characterizes a member that is primarily involved in morphogenesis, rather than in defense mechanisms of *C. albicans*.

76. Calcium mobilization to cell wall in *Neurospora crassa*. P.Maruthi Mohan and Naveena Lavanya Latha, Department of Biochemistry, Osmania University, Hyderabad – 500 007 (A.P.), India

Calcium was found in significant quantities (25%) on mycelial surface of *N. crassa*. Removal of surface-bound calcium by EGTA resulted in complete replacement until 75% of the intracellular calcium pool is depleted. Loss of intracellular calcium pool was observed in vacuolar and mitochondrial fractions. A 60% decrease in calcium mobilization was observed with metabolic inhibitor (azide), while channel blockers; verapamil and nifidipine inhibited this almost completely. Concanamycin-A, a specific inhibitor of vacuolar ATPase inhibited Ca mobilization by more than 90%. Neomycin a general inhibitor of IP3 signal transduction pathway also inhibited Ca mobilization completely. Vacuolar mutants of *N. crassa (vma-5)* with defect in Ca transport failed to mobilize Ca on to surface. Toxic metal ion bound to surface by proportionally displacing Ca and Mg ions. Scanning Electron microscopy showed gross structural changes on hyphal surfaces of EGTA treated mycelia as compared to controls. Transmission EM pictures showed increased number, size and fusion of vacuoles. Periplsamic marker enzymes (invertase and alkaline phosphatase) and small molecular weight cell wall proteins/peptides were released when mycelia were treated with EGTA. The implications of Ca mobilization on to the cell wall and its relation to toxic metal ion interactions at both surface and possible role of Ca in maintenance of cell wall structure will be discussed. Acknowledgments: The work was supported by grants from the Department of Science & Technology(PMM)and UGC-SAP(DRS-I)

77. Regulation of Asynchronous Mitoses within a Multinucleated Cell. Amy S. Gladfelter, Katrin Hungerbuehler, Hans Peter Helfer, and Peter Philippsen. Molecular Microbiology, University of Basel Biozentrum, Basel, Switzerland.

We use the filamentous fungus, Ashbya gossypii, as a model organism to study the regulation of nuclear division in multinucleate cells. In this organism, mitosis occurs asynchronously such that only 1 or 2 nuclei divide in a cell compartment with 6 or 7 nuclei. We have generated GFP labels to visualize nuclei, spindle pole bodies, and septins in live cells to analyze the dynamics and regulation of asynchronous mitotic events. Using these tools, we have determined that: 1.) All nuclei have the capacity to divide, 2.) Neighboring nuclei reside in different cell cycle

stages, and 3.) Mitotic frequency is highest at branch points. Furthermore, we have shown that septin proteins are arranged in elongated bar structures rather than rings as observed in other fungi. Interestingly, Swe1p, a wee1 homologue, may function to maintain this asynchronous nuclear division because swe1 mutants display increased nuclear density. We speculate that Swe1p is locally stabilized due to the distinct structure of the septin scaffold in these cells.

78. Requirements for formation of meiotic DNA double-strand breaks in *Coprinus cinereus*. Alexander M. Many, Sonia Acharya, and Miriam Zolan. Biology, Indiana University, Bloomington, IN

We are using the basidiomycete *Coprinus cinereus* to investigate genes that play a role in meiosis and DNA double strand break (DSB) repair. *Coprinus* features a naturally synchronous progression through the early stages of meiosis. We have identified a series of genes, mutant strains of which are sensitive to ionizing radiation and fail to complete meiosis. When cloned, two of these genes were found to encode members of the Mre11 complex, Mre11 and Rad50. This complex has been shown to be important for numerous activities in which an exposed DSB is present. This includes repair of DSBs after ionizing radiation, and the formation and processing of the meiotic DSB. Meiotic recombination requires a programmed DSB that has been found in *Saccharomyces cerevisiae* to be created by Spo11. We have cloned the *Coprinus spo11* gene, and isolated a mutant, *spo11-1*, which encodes a truncated*spo11* gene product lacking four of the five conserved domains. A *spo11-1* strain fails to complete meiosis, but progression through meiosis can be rescued by ionizing radiation-induced DSBs. The *rad50-4* strain *Coprinus* of undergoes an arrest in the diffuse diplotene stage of meiosis. The *spo11-1* strain of *Coprinus* exhibits a programmed cell death phenotype. A double mutant was made using these two strains, and it exhibits a *spo11-1* like phenotype. Upon irradiation in early prophase the double mutant reverts to a *rad50-4* like phenotype. This indicates that the formation of a meiotic DSB by Spo11 does not require wild -type Rad50 protein. To complement these studies an*mre11-1;spo11-1* double mutant is being constructed. *A mre11-1* strain of Coprinus undergoes meiotic arrest at a condensed metaphase I-like state, enabling an analysis similar to that performed for the *rad50;spo11* double mutant. Co-immunoprecipitation of the Mre11 complex and immunohistological studies of Mre11, Rad50, and Spo11 will also be carried out to gain further insight into the early meiotic roles of these genes.

79. The *snxA1* gene of *Aspergillus nidulans* affects the DNA damage checkpoint. Sarah Lea McGuire, Jim Goode, Allison McElvaine, and David Norris. Millsaps College, Jackson, MS, USA.

The*snxA1* mutation of *A. nidulans* was identified as an extragenic suppressor of the *nimX2*^{cdc2} mutation. In addition to this suppression, *snxA1*leads to a late G1/early S arrest at 20C. Protein kinase assays and western blots suggest that*snxA1* does not suppress the *nimX2* mutation by increasing the activity or protein level of NIMX. Double mutant analysis has shown that *snxA1* causes a synthetic lethal phenotype in combination with a deleted *ankA*^{wee1} and partially suppresses the *nimT23*^{cdc25} mutation. To determine if *snxA1* is involved in the DNA damage checkpoint response, we germinated *snxA1* mutant cells and then exposed them to 0.02% MMS at 32C and 44C to induce DNA damage. Under these conditions,*snxA1* mutants had a significantly elevated mitotic index, similar to checkpoint-deficient*bimE7* cells: at 32C, 28% of *snxA1* cells and 29% of *bimE7* cells were in mitosis, and at 44C 47% of *snxA1* cells and 55% of *bimE7* cells were in mitosis. In both cases, 0% of wild-type cells were in mitosis. These data suggest that *snxA1* leads to a DNA damage checkpoint defect. Western blots indicate that the level of Tyr15 phosphorylation of NIMX is decreased in *snxA1* cells compared to wild type cells exposed to MMS, suggesting that the checkpoint defect caused by *snxA1* ultimately functions through Tyr15 phosphorylation of NIMX. Supported by NIH R15GM55885.

80. Functional analysis of the *Aspergillus nidulans* HbrB protein. Gatherar, I. M.[1], Pollerman, S.[1], Dunn-Coleman, N.[2] and Turner, G.[1] [1]Department of Molecular Biology and Biotechnology, University of Sheffield, UK. [2]Genencor International, Palo Alto, CA. US.

An orphan gene specific to the filamentous fungi, *hbrB*, has been characterised. The gene was isolated by complementation of a temperature sensitive allele, *hbrB3*, which results in a hyperbranching phenotype. Though absent from yeasts, HbrB has apparent homologues in *N. crassa* and *A. fumigatus* (49% and 67% identity respectively). In order to study the gene function, a promoter exchange with the conditionally expressed gene,*alcA*, was undertaken. The *alcA* promoter is induced by ethanol, ketones and threonine and is repressed by glucose. Approximately 1 Kb of the 5' end of *hbrB* was cloned into the *alcA* expression vector, pAL3, which was used to

transform wild type *A. nidulans* (1). A Southern blot to confirm correct integration was carried out on the strains with a regulatable phenotype on glucose and threonine. One strain appeared to have the correct integration pattern. Downregulation of this promoter replacement strain gives a loss of polarity phenotype, which is different from that of the temperature sensitive phenotype. A sexual cross between the promoter replacement strain (alcA::hbrB) and the temperature sensitive mutant (*hbrB3*) showed that there was no recombination between *hbrB* and *hbrB3* indicating that both alleles are at the same locus. To determine cellular localisation of the *hbrB* product, the gene will be fused with the GFP (Green Fluorescent Protein) gene under control of the *alcA* promoter. This vector will be used to transform the *hbrB3* temperature sensitive strain in order to visualise the location of HbrB, and to confirm function of the GFP-HbrB fusion product through growth at restrictive and permissive temperatures. References

1. Waring, R. et al. (1989). Characterisation of an inducible expression system in Aspergillus nidulans using alcA tubulin-encoding genes. Gene, 79, 119-130.

81. Cell wall remodeling in response to cell wall stress in *Aspergilus niger*. R. A. Damveld[1], M. Arentshorst[1], F.M. Klis[3], C.A.M.J.J. van den Hondel[1,2] and A.F.J. Ram[1,2]. [1)]Leiden University, IMP, Leiden, The Netherlands, [2)]TNO-Nutrition, AMGT, Zeist, The Netherlands, [3)] Fungal Research Group, University of Amsterdam, Amsterdam, The Netherlands.

The fungal cell wall is a highly dynamic structure: both its composition and architecture respond to internal and external stimuli to ensure the integrity of the cell wall. Using *Aspergilus niger* as a model fungus we have studied how the fungus responds to the presence of an antifungal compound, Calcofluor White, which interferes with normal cell wall assembly. The addition of Calcofluor White (CFW) to just germinated spores resulted in a retardation of growth and swelling of hyphal tips. After 2 to 4 hours, depending on the concentration CFW used, growth resumed. Using PCR, we have isolated several *A. niger*genes that encode proteins involved in cell wall biosynthesis. Northern analysis indicated that the mRNA level of *agsA*, encoding an alpha-1,3-glucan synthase, and *gfaA*, encoding the glutamine:fructose-6-phosphate aminotransferase enzyme involved in de biosynthesis of UDP-*N*-acetyl-glucosamine, were induced 20-fold an 4-fold respectively. This finding suggests important roles for the cell wall polymers alpha-1,3-glucan and chitin in response to cell wall stress to ensure cell wall integrity.

82. Characterization of genes involved in the histidine kinase TcsB-HogA MAPK cascade in*Aspergillus nidulans*. Kentaro Furukawa, Keietsu Abe, Youhei Yamagata, and Tasuku Nakajima. Department of Molecular and Cell Biology, Graduate School of Agricultural Sciences, Tohoku University.

We have cloned and characterized a novel *Aspergillus nidulans* histidine kinase gene, *tcsB,* encoding a membrane-type two-component signaling protein homologous to the yeast osmosensor Sln1p, which transmits signals through the Hog1p-MAPK cascade in yeast in response to osmotic stimuli. Overexpression of the *tcsB* cDNA suppressed the lethality of the temperature-sensitive osmosensing-defective yeast mutant *sln1-ts*. However, *tcsB* cDNAs in which the conserved phosphorylation site His^{552} or phosphorelay site Asp^{989} had been substituted failed to complement the *sln1-ts* mutant. Introduction of the *tcsB* cDNA into the yeast double mutant*sln1-delta sho1-delta*, which lacks two osmosensors, suppressed lethality in high-salinity media and activated the HOG1 MAPK. These results imply that TcsB functions as an osmosensor histidine kinase. We constructed an *A. nidulans* strain lacking the *tcsB* gene (*tcsB-delta*) and examined its phenotype. However, unexpectedly, the *tcsB-delta* strain did not exhibit a detectable phenotype in either hyphal development or morphology on standard or stress media. The result suggests that *A. nidulans* has more complex and robust osmoregulatory systems than the yeast SLN1-HOG1 MAPK cascade. Functional analysis of other histidine kinase genes homologous to yeast *YPD1* and *SSK1* will be also discussed.

83. Characterization of *Aspergillus nidulans* alpha-subunits of heterotrimeric G-proteins and their role in the control of conidial germination and cAMP signaling. Anne Lafon(1), Mi-Hee Chang(2),Patrick van Dijck(3), Johan Thevelein(3), Kwang-Yeop Jahng(2), Christophe d'Enfert(1). (1)Institut Pasteur, France, (2) Chonbuk National University, South Korea, (3)K.U. Leuven, Belgium

In an effort to identify the cellular components involved in the activation of adenylate cyclase at the onset of *A. nidulans* condial germination, we have focused our interest on the role of 3 Galpha proteins, FadA, GanA and GanB. Phenotypic analyses have been performed with fadA, ganA and ganB null mutants and show that only the ganB

mutant is defective for trehalose breakdown and for the kinetics of germ tube formation, suggesting a direct role of GanB in the activation of adenylate cyclase. Attempts to complement through expression of ganA, ganB or fadA the defects that are observed in a *S. cerevisiae* strain with a null mutation in the GPA2 gene which encodes the Galpha subunit responsible for activation of adenylate cyclase in response to glucose were unsuccesful despite, in particular, the close relationship of GanB and Gpa2. We suggest that this inability of GanB to fulfill the functions of Gpa2 results from a defect in the interaction with either adenylate cyclase or the *S. cerevisiae* G-protein coupled receptor Gpr1 which acts upstream of Gpa2. Experiments aimed at testing this hypothesis will be presented. Physical interactions between GanB and adenylate cyclase are currently analyzed using two-hybrid experiments involving GanB, adenylate cyclase and a putative cyclase associated protein.

84. Cloning and characterization of two nucleotide excision repair genes, *ncRAD10* and *ncRAD14*, in *Neurospora crassa.* Akihiko Ichiishi, Masahito Sato, Takaharu Niki, and Makoto Fujimura. Dept of Life Sciences Toyo University, Izumino, Japan.

In *Neurospora crassa*, nucleotide excision repair (NER) mechanism has not been well characterized in molecular level. To study NER mechanism in *N. crassa*, we tried to clone *Neurospora* genes involved in NER. In searching the genome database of *N. crassa*, several genes which homologues to *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* NER genes were found. Two genes encoding NER genes homolog, *ncRAD10* and *ncRAD14*, were isolated by PCR. The amino acid sequence of ncRad10p showed 48% of identities to the *S. pombe* Swi10 protein which was homologues to *S. cerevisiae* Rad10p and human ERCC1 protein. The predicted amino acid sequence of ncRad14p showed 33% homology to *S. cerevisiae* Rad14p with a well conserved zinc finger motif. To characterize the function of *ncRAD10* and *ncRAD14*, we disrupt these two genes by the RIP. Both RIP mutants showed sensitivity to UV light and 4-NQO but not to MMS on a spot test. This phenotype is similar to that of*mus-38* mutant which is defective in NER in *N. crassa*.

85. Interaction of mitochondria with microtubules in the filamentous fungus*Neurospora crassa.* Florian Fuchs, Walter Neupert and Benedikt Westermann. Institute for Physiologcal Chemistry, Ludwig-Maximilians-University Munich,

The establishment and maintenance of the three-dimensional structure of eukaryotic cells depends on active transport and positioning of organelles along cytoskeletal elements. The biochemical basis of these processes is only poorly understood. We analysed the interaction of mitochondria with microtubules in the filamentous fungus *Neurospora crassa*. Mitochondria were fluorescently labelled by expression of matrix-targeted green fluorescent protein (mtGFP). Upon isolation, mitochondria collapsed to round spherical structures that were, however, still able to interact with microtubules in vitro. Binding of mitochondria to microtubules was dependent on peripherally associated proteins on the organellar surface, and was sensitive to adenine nucleotides. MMM1, a mitochondrial outer membrane protein important for maintenance of normal mitochondrial morphology, was not required. This suggests that the interaction of mitochondria with the cytoskeleton is independent of MMM1. Furthermore we detected a novel kinesin-related protein on mitochondria and outer membrane vesicles, which might be a mediator of the interaction. Taken together we conclude that mitochondrial morphology is maintained by a complex interplay of extrinsic and intrinsic factors, including ATP-dependent proteins on the organellar surface.

86. PHOA interacting proteins are involved in regulation of development in *Aspergillus nidulans*. Dongliang Wu, Shahr B. Hashmi, Xiaowei Dou, and Stephen A. Osmani Department of Molecular Genetics, The Ohio State University, Columbus, OH43210, USA

PHOA was identified as a non-essential cyclin-dependent kinase which controls the developmental program of Aspergillus nidulans in an environment dependent manner (Bussing and Osmani, EMBO J 1998 17, 3990). Four genes interacting with PHOA have been isolated by a yeast two-hybrid system and have been provisionally named pipA-C pipD (PHOA interactive protein A-C D). In vitro transcription, translation and coimmunoprecipitation results confirmed the physical interactions between PHOA and PIPA, PIPB, PIPD proteins. We used the pyroA marker gene to replace the four structural genes and got the knockout mutant of pipA and pipB which were viable indicating that these genes are not essential. Deletion of pipA (which encodes a potential transcription factor) caused some phenotypes similar to that caused by deletion of phoA, suggesting pipA may be involved in regulating development downstream of phoA. pipB encodes a PCL-like cyclin and its deletion caused increased conidiation but

lack of sexual sporulation when compared to wildtype or phoA deleted strains when grown under different growth conditions. The data indicate that this PCL-like cyclin protein may repress asexual development but be necessary for sexual development. Deletion of pipC could not be achieved in normal haploid cells but could be maintained in heterokaryons carrying some nuclei with deleted pipC and others with wild type pipC. Conidia generated from these heterokaryons were unable to germinate under selective conditions indicating that deletion of pipC causes lethality and an inability to germinate. Sequence analysis indicates that pipC encodes a recently isolated helix-loop-helix transcription factor termed AnBH1 (Caruso et al., 2002 J. Mol. Biol. 323, 425). Finally, pipD encodes another cyclin like protein with similarity to yeast Pho80 and this gene has not been successfully deleted yet. As expected, we have been able to identify cyclin-like partners for the PHOA cyclin dependent kinase and also potential transcription factors with which PHOA may interact. Importantly, deletion analysis indicates that some of these genes play roles during development. Further analysis of PHOA and its interactive proteins should shed further light on how the environment can modify development in multicellular eukaryotes.

87. Localization of recombinant gene products in mycelial fungi by means of *egfp*reporter plasmids.
Mayrhofer S, Hoff B, Masloff S, Kück U, Pöggeler S. Department for General and Molecular Botany, Ruhr-University Bochum, 44780 Bochum, Germany

In recent years, the *gfp* gene encoding the green fluorescent protein (GFP) has become an important reporter gene for *in vivo* studies. The development of the enhanced *gfp* gene (*egfp*), which is adapted to altered codon usage in mammals, make the efficient expression of this reporter gene in ascomycete fungi possible. Here, we describe the construction and application of a series of plasmids, which support the expression of the *egfp* gene in the two ascomycetes *Sordaria macrospora* and *Acremonium chrysogenum*. The vectors assist the study of diverse developmental processes. The plasmids presented here, include a promoterless *egfp* vector for monitoring the expression of cloned promoters/enhancers in fungal cells, and vectors for creating translation fusions to the N-terminus of EGFP. The vectors were further modified by introducing a variant hygromycin B phosphotransferase (*hph*) gene, lacking the commonly found *Nco*I site. Instead, the single *Nco*I site, containsing an ATG start codon, can be used for cloning translational fusions in front of the *egfp* gene. The applicability of these vectors is demonstrated by analysing transcription regulation as well as protein localization and secretion in the two ascomycetes *Acremonium chrysogenum* and *Sordaria macrospora*. In the latter, the heterologous *egfp* gene is stably inherited during meiotic divisions as can easily be seen from fluorescent ascospores.

88. The role of myosin I during hyphae formation in *C. albicans*.
U. Oberholzer, T. Iouk and M. Whiteway. Biotechnology Research Institute, Montreal, Canada.

Myosin I is required for hyphal formation in the pathogenic yeast *Candida albicans*, due to its role in organizing the cortical actin patches and hence its role during endocytosis. We have investigated the role of myosin I tail domains and found that the putative membrane binding domain TH1 and the calmodulin interacting IQ motifs are required for optimal vegetative growth, for hyphae formation and for fluid phase endocytosis. Deletion of these domains allows the formation of pseudohyphae but not of hyphae. Moreover, deletion of either domains leads to depolarized cortical actin patches in these pseudohyphal structures and the corresponding myosin-GFP fusion proteins are mislocalized, i.e. they are not found in patches at the pseudohyphal tip. In contrast, we found that the SH3, acidic A- or ATP independent actin binding domain TH2 are not required for the formation of hyphae. Consistent with this observation, the actin cytoskeleton of these hyphae is not affected by the deletion of the SH3 or A- domains, and the localization patterns of the corresponding myosin-GFP fusion proteins appear normal. Surprisingly, cortical actin patches are depolarized in the hyphae where the TH2 domain of myosin I is deleted. Moreover, fluid phase endocytosis is defective in this mutant suggesting that defective and depolarized cortical actin patches are not essential for hyphae formation. Finally, in all of these mutants except in those where the SH3 or A domains have been deleted, Arp3-GFP patches are depolarized, i.e. are not localized in the hyphal tips. We propose a model for how hyphae formation in *C. albicans* is dependent on myosin I.

89. A role for the Ubiquitiin Conjugating Enzyme, Ubc4, in G2 and Mitosis in *Aspergillus nidulans*.
Peter Mirabito, Kuttalaprakash Chudayalandi, and Alberto Ribes-Zamora. Dept of Biology, University of Kentucky, Lexington, KY.

The long-term goal of this research is to determine the mechanisms regulating mitosis in *Aspergillus nidulans*. Previous work demonstrated that *Aspergillus* Anaphase-Promoting Complex (aka Cyclosome or APC/C) has a novel role in G2 in addition to its well-conserved function in mitosis and G1. As part of our investigation of APC/C regulation in G2, we have identified a recessive lethal mutation in *nadH*, the *A. nidulans* UBC4 gene, which phenocopies APC/C mutations in G2 and mitosis. nadH^{UBC4} is required for chromosome segregation but not chromosome decondensation, whereas APC/C is required for both. This suggests that NadH^{Ubc4} acts with APC/C in G2 and in chromosome segregation, whereas another ubiquitin-conjugating enzyme acts with APC/C in chromosome decondensation. These results suggest ubiquitin-conjugating enzymes may play a role in the specificy of APC/C function.

90. Depletion of a polo-like kinase in *Candida albicans* activates hyphal-like growth and transcription. Catherine Bachewich (1), David Thomas (2), Malcolm Whiteway (3). (1) Health Sector, Biotechnology Research Institute (BRI), National Research Council of Canada (NRC), Montreal, Canada; (2) Department of Biochemistry, McGill University, Montreal, Canada; (3) Health Sector, BRI/NRC, Montreal, Canada

Morphogenesis in *Candida albicans* is an important virulence-determining factor, since the environmentally-stimulated switch between yeast and hyphal growth can increase pathogenesis. We identified *CaCDC5*, a cell cycle regulatory polo-like kinase in*Candida albicans*, and demonstrate that shutting off its expression induced cell cycle defects and dramatic changes in morphology. Cells lacking CaCdc5p were blocked with short spindles and unseparated chromatin. GFP-tagged CaCdc5p localized to unseparated spindle pole bodies, the spindle, and chromatin, consistent with a role in spindle formation. The cell cycle defects were accompanied by the formation of hyphal-like filaments under yeast growth conditions. Similar spindle defects and a corresponding induction of filaments occurred when yeast cells were exposed to hydroxyurea, suggesting a link between spindle function and filament formation. The filaments resembled serum-induced hyphae with respect to many cytological features, and microarray analyses demonstrated that the filaments expressed several factors normally modulated in serum-induced hyphae. Filament formation required CaCdc35p, but not Efg1p or Cph1p, of the hyphal-signaling pathways. Thus, an internal cell cycle-related cue can activate hyphal regulatory networks in *Candida*.

91. Cdc42 regulates cytokinesis and morphology in *Ustilago maydis.* Michael Mahlert, Leonora Leveleki, Björn Sandrock and Michael Bölker, Dept. of Biology, University of Marburg, Karl-von-Frisch-Strasse 8, D-35032 Marburg, Germany,

Small ras-like GTPases are known to act as molecular switches regulating diverse processes such as cytoskeleton organization, cell cycle and vesicular traffic. In *U. maydis*, the GTP binding protein Cdc42 plays a central role in regulating cytokinesis and cell polarity. A signalling module triggers cell separation and consists of a Rho/Rac-GEF (Don1), which activates Cdc42, and a Ste20 like kinase (Don3), which is likely to be a target for Cdc42. We could show that expression of the dominant active version Cdc42-Q61L rescues the cytokinesis defect of *don1* mutants but not that of *don3* confirming the role of Cdc42 in this signalling pathway. Expression of dominant negative Cdc42-T17N in wild type cells leads to deformation of cells and lateral budding. This demonstrates that Cdc42 plays an additional role in polarized growth of *U. maydis*. We could identify another Ste20 like kinase, Cla4, which regulates in a parallel Cdc42 containing pathway polarized growth and septum formation. We were able to generate a deletion mutant for *cdc42*, which surprisingly is viable. Depending on the genetic background, the mutants exhibit either only a cell separation defect or additional morphological aberrations. Since the phenotype of a conditional *cdc42* mutant strain is less pronounced, even a low level of Cdc42 expression may be sufficient to maintain a proper cell shape.

92. Cell wall synthesis in *Aspergillus nidulans*. Gerald Hofmann[1], Mhairi McIntyre[1], and Jens Nielsen[1]. [1]Center for Process Biotechnology, BioCentrum-DTU, Denmark.

Since the beginning of fungal research the cell wall of fungi has been subject of many studies. Despite these efforts, our knowledge about the synthesis and the regulation of its composition is still incomplete. One important step in the synthesis of cell wall compounds is the formation of the precursor UDP-glucose, since this metabolite is used in the anabolism of glucans, and as glucosyl donor for the glycosylation of proteins incorporated in the cell wall. Furthermore this step is involved in the catabolism of galactose, and the formation of trehalose. Therefore the gene *ugpA*, encoding the enzyme UDP-glucose pyrophosphorylase (EC 2.7.7.9), was cloned from *Aspergillus nidulans*

and characterised. The derived protein sequence comprises 514 amino acids and shows a very high degree of conservation to other eukaryotic UDP-glucose pyrophosphorylases. *In silico* promotor analysis revealed several putative regulatory elements, including binding sites for CreA, PacC, and one site that might be recognised by a homologue of the Gal4 protein from *S. cerevisiae*. Northern blott analysis was carried out to further investigate possible regulation through these sites. These results and further characterisation of the gene and its role in the cell wall metabolism, which are currently underway, will be presented.

93. The kinesin motor KipA is required for polarized growth in *Aspergillus nidulans.* Sven Konzack, and Reinhard Fischer, Max-Planck-Institute for terrestrial Microbiology, Karl-von-Frisch-Str., D-35043 Marburg, Germany

Motor proteins are key factors in various dynamic processes in eukaryotic cells. To analyze organelle movement over long distances, we are studying the function of different kinesins in*A. nidulans*. So far two mitotic kinesins, BimC and KlpA were described. Taking advantage of the genomic DNA sequencing project at Cereon Genomics, gene fragments corresponding to four new kinesins, designated KifA, KipA, KipB and KinA, were identified. The further results obtained for KipA will be presented here. KipA displayed highest homology to KIF3C from rat, which is involved in vesicle transport, and to Kip2p that is involved in nuclear migration and spindle orientation. KipA was predicted to form a dimer, and a KipA-GFP fusion protein was predominantly found in the cytoplasm. After ATP-depletion of the cell (uncoupling with CCCP), a fraction of KipA localized to cytoplasmic microtubules. In order to assign a function to KipA, we deleted the motor domain of KipA from the genome of *A. nidulans* and studied the dynamics of microtubules, mitochondria and nuclei by GFP technology. We did not detect any differences in those processes in comparison to wt-strains. However, we observed a strong effect on polarized growth. Whereas the radial colony extension rate appeared similar to wild type, the directionality of the hyphae in *kipA* mutants was dramatically changed. Hyphae displayed a meandering growth phenotype, suggesting an effect of the kinesin deletion on the positioning of the Spitzenkorper. To further analyse the roles of KipA and possible overlapping functions with other motors, we constructed double and multiple mutations of KinA, KipA, KipB and Dynein. The analyses of their phenotypes are on the way.

94. Oxidative stress-induced Ca^{2+}-signaling in *Aspergillus nidulans* may be required for survival. Diana Bartelt, Mitra Singh, and Vilma Greene; Department of Biological Sciences, St. John's University, Jamaica, NY 11439.

The effects of oxidative stress on levels of calcium ion (Ca2+) in *Aspergillus nidulans* were measured using strains expressing aequorin in the cytoplasm ($Aeq_{cyt\ mt}$) [Greene, V., H. Cao, F. Schanne, and D. Bartelt. Cell. Signal. 14:437-443 (2002)]. When oxidative stress was induced by exposure to 10 mM H_2O_2, the mitochondrial calcium response (Ca^{2+}_{mt}) was greater than the change in cytoplasmic calcium (Ca^{2+}_{c})and was dose-dependent,while the increase in [Ca^{2+}_{c}] did not change with increasing [H_2O_2]. Ruthenium red (RR) blocked the increase in [Ca^{2+}_{mt}]. Eighteen hour cultures of *A. nidulans* survived 30 min exposure to 100 mM H_2O_2 and, following exposure, conidiated normally when grown overnight in YG medium. Treatment with RR alone had no effect on growth and development. Pretreatment with RR prior to 100 mM H_2O_2 exposure, severely inhibited growth and conidiation. Cytoplasmic and mitochondrial fractions were prepared from cells exposed to H_2O_2,without and with RR pretreatment. ATP levels decreased, and isocitrate dehdrogenase (IDH) activity was induced in extracts of cells exposed to H_2O_2 alone. Pretreatment with RR decreased the loss of ATP and inhibited the activation of IDH. (Supported by NIGMS R15 GM52630)

95. Functional analysis of *pre-1*, a pheromone receptor gene of *Neurospora crassa.* Hyojeong Kim and Katherine Borkovich. Department of Plant Pathology, University of California, Riverside, CA 92321

Two putative pheromone receptor genes for the heterothallic ascomycete *N. crassa*, *pre-1* and *pre-2*, were identified during BLAST searches against the entire genome database at the Whitehead Institute Center for Genome Research. The encoded proteins belong to the seven-transmembrane class of G-protein-coupled receptors and show significant sequence similarity to the a-factor receptor Ste3p and alpha-factor receptor Ste2p of *Saccharomyces cerevisiae*, respectively. Northern analysis showed that the genes were preferentially expressed under mating conditions in a mating type-specific manner. Delta *pre-1* mutants have been isolated and characterized in both mating types. They displayed normal vegetative growth, including hyphal fusion. During sexual development, they underwent normal

protoperithecial formation and were fertile as males. However, *mat A pre-1* strains were completely unable to initiate mating as females in crosses. The female sterility of the *mat A pre-1* mutant could be complemented *in trans* by wild type *pre-1*$^{+}$, but not by an activated allele of *gna-1*. The levels of all G protein subunits were unaffected in *mat A pre-1* strains. Thus, the pheromone receptor gene, *pre-1*, is essential for the fertilization of *mat A* strains, consistent with a role in initiating the pheromone response signaling pathway.

96. Mutations in *snoA* and *snoB*(suppressor-of-*nimO*) act through different mechanisms to rescue defects in the initiation of DNA synthesis. Steven W. James[1], Matthew J. O'Connell[2], Syef M. Hoque[1], and Brian P. McCarthy[1]. [1]Department of Biology, Gettysburg College, Gettysburg, PA USA. [2]Peter MacCallum Cancer Institute, East Melbourne, AUS.

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 unwinding through phosphorylation of DNA helicase subunits. Several approaches are underway to investigate *nimO* and*cdc7* function in *Aspergillus*. For example, *cdc7*^{asp} was isolated and, by two-hybrid analysis, cdc7p^{asp} was shown to physically associate with nimOp. Additionally, we generated partial *nimO*18 suppressors in two loci, *snoA* and *snoB* (suppressor-of-*nim**O*). *snoA/B*alleles partially alleviate the temperature sensitivity of *nimO*18 by restoring growth at 37ºC but not 43ºC. *snoA*31 and *snoB*59 do not interact genetically with ts-lethal mutations in any of 20 cell cycle control genes in the nim (*n*ever-*i*n-*m*itosis) and bim (*b*locked-*i*n-*m*itosis) collection. Thus, the suppressors appear to modify only *nimO* activity or function. The suppressors were tested in an ethanol-dependent, glucose-inviable strain carrying *alcA::nimO* as the sole *nimO* gene copy. Recessive *snoA* alleles rescued growth on glucose, suggesting that *snoA* mutations may bypass the requirement for*nimO*. However, by using a parasexual assay we found that instead of bypass, *snoA* alleles act by stabilizing or enhancing nimOp produced during leaky*alcA::nimO* expression on glucose. Conversely, semi-dominant *snoB* alleles could not rescue growth on glucose, suggesting that snoBp and nimOp may associate directly. This hypothesis is supported by the discovery that *snoB*59 harbors a missense mutation in the *cdc7*^{asp} gene. Sequencing of *cdc7*^{asp} from additional *snoB* alleles is underway to determine if *snoB* =*cdc7*^{asp}. (Supported by NSF-RUI# 01-14446 and by a Research and Professional Development Grant from Gettysburg College).

97. A Neurospora mannosyltransferase required for normal morphology and anastomosis. Amy Marie Piwowar, Shaun M. Bowman, Maria Ciocca, Mashel S. Al Dabbous, Gregory O. Kothe and Stephen J. Free. Dept. of Biol. Sciences, SUNY University at Buffalo, Buffalo, NY.

We have isolated and characterized three mutants affected in a cell wall biosynthetic mannosyltransferase. The levels of cell wall mannose and galactose are reduced by approximately 50% in the mutant cells. The mutant cells grow with an altered hyphal morphology which gives rise to a colonial growth pattern. The mutants are unable to produce normal macroconidia. We have found that the mutants are also affected in the ability to undergo anastomosis (hyphal fusion). The results demonstrate that the mannogalactan portion of the Neurospora cell wall is required for normal growth, development and cell fusion events.

98. The nuclear pore complex component SONB is involved in mitotic regulation and a DNA damage response pathway in *Aspergillus nidulans*. Colin De Souza and Stephen Osmani. Department of Molecular Genetics, Ohio State University, 802 Riffe Building, 496 W 12th Ave Columbus OH 43210.

The NIMA kinase is essential for mitotic entry in *Aspergillus nidulans* and is required for nuclear localization of mitotic regulators. We have isolated two nuclear pore complex (NPC) proteins as suppressors of the *nimA*1 temperature sensitive allele which normally arrests with uncondensed DNA, interphase microtubules and cytoplasmic NIMA. These proteins, SONA and SONB, physically interact and are apparent at the nuclear periphery during interphase but remarkably disperse upon mitotic entry before localizing once again at the nuclear periphery during mitotic exit. We also find that NIMA itself localizes to the nuclear periphery during mitotic entry and that expression of dominant negative NIMA constructs enhances this localization. We propose NIMA regulates mitotic entry by promoting SONA and SONB dissociation from the NPC (see also De Souza and Osmani Wed Mar 19). SONB is a member of the human NUP98/NUP96 family of nucleoporins. Interestingly, at 42ºC, the *sonB*1 allele also displays a remarkably high sensitivity to agents which induce double strand DNA breaks, but less sensitivity to

DNA damage induced by UV irradiation which does not cause double strand breaks. We provide evidence that this DNA damage sensitivity is not due to loss of checkpoint regulation over mitotic entry. We will also describe an extra copy suppressor screen aimed at identifying genes genetically interacting with SONB in response to DNA damage.

99. Orp-1, an Opsin Related Protein of *Neurospora crassa*. Marek Nemcovic and Katherine A. Borkovich Department of Plant Pathology, University of California, Riverside, CA 92521

The *Neurospora crassa* ORP-1 protein is a member of a newly established class of seven transmembrane proteins termed Opsin related proteins or ORPs. These proteins exhibit significant homology to archaeal rhodopsins, but are missing critical residues in the retinal binding pocket. Fungal ORPs appear to function in cellular responses to pH, organic solvents and stress signals. Here, we report a role for ORP-1 in stress responses. Sequence analysis revealed a HSE and an AP-1 response element in the promoter of the *orp-1* gene. Western and Northern analysis support regulation of *orp-1* expression by heat shock. The presence of the AP-1 element suggests that ORP-1 is under general amino acid control (cross-pathway amino acid starvation control). Treatment of the *orp-1* deletion mutant with 3-aminotriazole resulted in reduced growth compared to wild type.

100. A novel G-protein coupled receptor family identified in *Neurospora crassa*. Krystofova S., Borkovich KA. University of California Riverside, Department of Plant Pathology.

A family consisting of three G-protein coupled receptors (GPCRs) named GPR-1, GPR-2 and GPR-3 has been identified in both the MIPS and the Whitehead Institute genome databases of Neurospora crassa. The encoded proteins are highly conserved in the seven transmembrane helices, and homology searches show similarity to the Family 2 of GPCRs. Phenotypic analysis of a *gpr-2* null mutant did not reveal any particular defects, suggesting possible redundancy. This study is currently focused on disruption of *gpr-1* and *gpr-3* and characterization of multiple mutants in the *gpr-1, gpr-2* and *gpr-3* genes.

101. Characterization of the Vo Sector of the *Neurospora crassa* Vacuolar ATPase. Christopher L Chavez, Kimberly H. Haw, Emma Jean Bowman, and Barry Bowman, U. of California , Santa Cruz, CA 95064

The vacuolar ATPase is a large multi-subunit 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 Vo sector of the enzyme is embedded within the membrane and contains the proton pore. At least four different genes (*vph-1, vma-3, vma-11,* and *vma16*) encode for proteins which make-up the Vo sector. In an effort to elucidate the role(s) of these gene products in V-ATPase function, knockouts of the genes were sought. *vph-1, vma-11,* and *vma-16* mutant strains were generated via RIP (Repeat-Induced-Point-Mutation) and modified RIP protocols. Due to meager spore germination rates, these mutants were extremely difficult to isolate. The RIP procedure failed to yield any viable strains in which the *vma-3* gene was inactivated. A *vma-3* mutant was eventually isolated using homologous gene-replacement in a heterokaryotic host. 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. The major phenotype difference between strains with different V-ATPase mutations is the viability of ascospores. The observation that some subunits are essential for ATPase activity and for ascospore viability suggests that some of the gene products may have additional functions.

102. MesA, a novel fungal protein required for localized cell wall deposition at hyphal tips. Claire Pearson and Steven D. Harris, Plant Science Initiative and Department of Plant Pathology, University of Nebraska, Lincoln, NE, 68588-0660.

In *Aspergillus nidulans*, SepA is required for septum formation and polarized hyphal extension. SepA is a member of the conserved formin family of proteins, which mediate the formation of actin filaments in response to cellular signals. We have previously shown that SepA functions interdependently with actin to form a contractile ring at septation sites. In addition, SepA displays dynamic localization to hyphal tips. However, the observation that *sepA* mutants can still establish hyphal polarity and recruit actin to hyphal tips suggests that a parallel pathway contributes

to these functions. To identify components of this pathway, we conducted a screen for mutations that enhance the characteristic morphological defects of *sepA* mutants. Our screen yielded four mutants, *mesA-D*. The most dramatic synthetic phenotypes are observed in *mesA1 sepA* double mutants, which at higher temperatures, accumulate as large swollen spores filled with nuclei. Even in an otherwise wildtype background, *mesA1* mutants display defects in the maintenance of hyphal polarity. Analysis of *mesA* mutants revealed that the aberrant morphology is most likely caused by an inability to properly localize spatial determinants required for localized cell wall deposition at the hyphal tip. For example, the normal pattern of SepA::GFP localization at hyphal tips is dramatically perturbed in *mesA1* mutants. Molecular characterization of MesA shows that it is a potential transmembrane protein, with homologues found only in other fungi. We propose that MesA functions as a morphological landmark that specifies sites of localized cell wall deposition and recruits the fungal analogue of the polarisome

103. Patterns of germ tube emergence and cell wall deposition in germinating *Fusarium graminearum* macroconidia. Steven D. Harris, Plant Science Initiative and Department of Plant Pathology, University of Nebraska, N234 Beadle Center, Lincoln, NE, 68588-0660.

The fungus *Fusarium graminearum* (teleomorph *Gibberella zeae*) is the causative agent of wheat and barley scab. This disease is initiated and spread via the airborne dispersal of ascospores and macroconidia, respectively. Our ultimate goal is to identify cell wall proteins from *F. graminearum* spores that are dynamically expressed and play a functional role in host colonization. Towards this goal, we have first characterized the patterns of germ tube emergence and cell wall deposition in germinating macroconidia. *F.graminearum* macroconidia typically contain three to seven septated cells. When germinated on rich, sucrose-based media, the first germ tube emerges from one of the pole cells (<90% of the time) in a manner that is independent of nuclear division. In contrast, although internal cells undergo isotropic swelling and nuclear division, they typically produce the first germ tube only if the pole cell is dead or the macroconidia is subjected to stress. The use of fluorescent lectins and other cell wall probes revealed a specific pattern of cell wall deposition in germinating macroconidia. This was most obvious when chitin deposition was examined using FITC-conjugated wheat germ agglutinin. Finally, we have used a combination of 2D gel electrophoresis and concanavalin-A binding assays to identify candidate cell wall proteins that display dynamic expression patterns during macroconidial germination. We propose that these proteins are likely to interact with plant surface components, and thereby play important roles in mediating host colonization.

104. Patterns of Actin Distribution in *Neurospora crassa* Actin Mutants. Aleksandra Virag, Anthony J.F. Griffiths. University of British Columbia, Botany Department, Vancouver, BC, Canada

Actin is necessary for hyphal morphogenesis, and actin filaments are laid down at sites of tip growth and branch emergence. It is not clear what factors command the localization and dynamics of actin at the hyphal tip. These factors are most likely a part of the machinery that regulates hyphal morphogenesis. By disrupting the actin gene in *Neurospora crassa* its mode of involvement in hyphal growth and branching can be deduced. *N. crassa* actin mutants were generated by either UV mutagenesis or RIP (repeat induced point mutations), and caused various morphological abnormalities. Actin filaments were visualized in these actin mutants by immuno-fluorescent labeling. A range of different actin patterns was detected at hyphal tips of the actin mutants. Some of these patterns include an actin distribution similar to the wild type with a mainly round apical actin dot and a peripheral network of smaller actin dots, absence of the apical actin dot, and two or multiple apical actin dots. The results presented indicate a significant role of actin in the shaping of hyphae and their branching.

105. Comprehensive expression analysis of the cell wall related genes in *Aspergillus oryzae* using the disruption mutant of protein processing enzyme gene (*kexB*). Osamu Mizutani, Tomonori Fujioka, Youhei Yamagata, Keietsu Abe, and Tasuku Nakajima. Molecular and Cell Biology, Tohoku University, Sendai, Japan

KexB is a processing proteolytic enzyme in fungi. To understand the involvement of KexB in protein processing, we cloned and disrupted *kexB* gene (*DkexB*) in A. oryzae. The *DkexB* strain formed small and shrunk colonies with significantly poor conidia generation on the Czapek-Dox (CD) agar plate. It also showed hyper-branched mycelia in liquid culture. When *DkexB* was cultivated under the various environmental conditions, we noticed that the phenotypes of the *DkexB* were restored under high osmolarity in both solid and liquid culture conditions. As a result of the observation of the phenotypes, we speculated that KexB might play an important role in processing of the gene products related to branching and conidia formation in solid cultivation. Then, to investigate the role of KexB

in the expression of morphogenesis related genes in *A. oryzae*, we performed comprehensive gene expression analysis between the *DkexB* and the wild-type by using DNA microarrays containing 2,000 *A. oryzae* cDNAs. As a result, expression levels of ca.300 genes in the *DkexB* were significantly higher than the corresponding genes in the wild-type under solid culture conditions. In particular, the expression levels of *chsB*, *chsC* and *gel2*, which encode the cell wall synthesis enzymes, increased in the *DkexB*. Expression levels of the cell wall biosynthesis related genes in various growth stages will be also compared between the *DkexB* and the wild-type.

106. Characterization of two PAK kinases of *Pneumocystis* and their interaction with Rho-type GTPases. Nichole Heubner, Reiko Tanaka, George Smulian. University of Cincinnati College of Medicine, Cincinnati VA Medical Center, Cincinnati, Ohio

P21 associated kinases (PAKs) are important components of many signal transduction pathways involved in transmitted extracellular signals via MAP kinase cascades and involved in cytoskeletal rearrangement. Two PAKs have been identified in the opportunistic fungal pathogen, *Pneumocystis carinii*. One gene, identified in a cluster of signal transduction genes, was named *ste20* due to apparent homology with the Ste20 gene of*Saccharomyces cerevisiae*. Pc*ste20* is predicted to encode a protein containing both a plecstrin homology (PH) domain, predicted to be involved in membrane localization, and a Cdc42/Rac Interactive Binding (CRIB) domain. A secong PAK gene, Pc*pak1*, is predicted to encode a p21-associated kinase containing a CRIB domain but lacking a PH domain. On review, *pak1* has greater homology to Ste20-like proteins while PcSte20 has greater homology to Cla4-like PAKs. In higher eukaryotes, 3 classes of Rho-type GTPases are found; Rho, Rac and Cdc42. Model fungal organisms such as *S. cerevisiae* and*Schizosacharomyces pombe* encode only Rho and Cdc42 homologs and among fungi, a Rac protein has only been identified in *Yarrowia lipolytica*. *P. carinii* expresses both Cdc42 and Rac1 homologs. Both Rac1 and Cdc42 were shown capable of interacting with the CRIB domains of PcSte20 and PcPak1 in vitro. Full characterization of the interaction of the PAKs and their cognate GTPase will shed light on the function of these proteins in *P. carinii*.

107. Genetic and cell biological analysis of cell morphogenesis in *Ustilago maydis*. Flora Banuett and Wei Wei. Department of Biological Sciences, CSULB, and Department of Biochemistry and Biophysics, UCSF.

Ustilago maydis is a Basidiomycete fungus that exhibits two basic vegetative morphologies: a haploid, yeast-like form that divides by budding and is non pathogenic and a filamentous dikaryon that grows at the tip and is pathogenic. A third form, the teliospore is not capable of vegetative growth but undergoes meiosis to produce the yeast-like form. The filamentous form arises by cell fusion of haploid cells of opposite mating type. This form grows in the plant and induces formation of tumors. The teliospore is produced only within the plant and results from execution of a discrete developmental program that takes place within the tumors. This program is characterized by karyogamy, hyphal fragmentation to produce cylindrical cells that undergo cell rounding, and deposition of a specialized cell wall around the spherical cells to produce mature teliospores. These morphological transitions are likely to involve changes in the organization of the cytoskeleton, which in turn may in part be directed by proteins that control cell polarity and cell wall remodelling events. We are interested in understanding the molecular mechanisms that underlie these morphological transitions. In order to identify some of these components, in particular proteins that organize the cytoskeleton, we have undertaken a multipronged approach involving genetic, cell biological, and biochemical techniques. We carried out a genetic screen to identify temperature-sensitive mutants that exhibit altered cell or colony morphology at the restrictive temperature. We identified approximately 80 mutants, 12 of which have been characterized further. Genetic analysis indicates that each mutant carries a single recessive mutation. The mutants exhibit additional phenotypes with respect to cell wall, sensitivity to drugs, and osmoticum. In addition, the mutants show altered organization of the microtubule or actin cytoskeletons. Here we present the results of the initial characterization of these mutants.

108. Genome defense by mutation and DNA methylation in Neurospora. Michael Freitag, Lakshmi Nimmagadda, Rebecca Williams, Gregory O. Kothe and Eric U. Selker, University of Oregon, Eugene.

During sexual development, Neurospora inactivates genes in duplicated DNA segments by a hypermutation process, RIP (repeat-induced point mutation). RIP introduces C:G to T:A transition mutations and creates targets for subsequent DNA methylation in vegetative tissue. The mechanism of RIP and its relationship to DNA methylation are not fully understood. Mutations in *dim-2*, a gene encoding a DNA methyltransferase responsible for all known

cytosine methylation in Neurospora do not prevent RIP. We disrupted a second putative DNA methyltransferase gene and tested mutants for defects in DNA methylation and RIP. No effect on DNA methylation was detected but the mutants showed recessive defects in RIP. Duplications of several marker genes were stable in crosses homozygous for the mutated potential methyltransferase gene, which we call *rid* (RIP defective). The same duplications were inactivated normally in heterozygous crosses. The *rid* genes of various *Neurospora* species are conserved. We produced full-length or truncated recombinant *N. crassa* RID in *E. coli* to assay for DNA methyltransferase activity. (Supported by NIH grant # GM35690 to E.U.S.)

109. Biology of Living Fungi. Patrick C. Hickey and Nick D. Read. Fungal Cell Biology Group, Institute of Cell and Molecular Biology, University of Edinburgh, King's Buildings, Edinburgh, EH9 3JH, UK.

A website and CD-ROM have been compiled which illustrate key aspects of the cell biology of living fungi, visualised at high spatial resolution by confocal microscopy. The aims are to provide a valuable resource for mycologists and a powerful educational tool for schools and universities worldwide. Novel techniques for imaging living fungal hyphae and cells have been developed in our laboratory using confocal microscopy. This advanced imaging technology involves the use of a wide range of vital fluorescent dyes and GFP probes, with particular care to minimise cellular perturbation and provides a unique insight into the biology of living fungi. Examples of movies that we will provide include: hyphal tip growth, branching, septum formation, organelle dynamics, mitosis, and 3-D reconstructions of spores and reproductive structures. The movies are accompanied by simple explanatory text. This resource will be accessible through the internet, complemented by a CD-ROM containing extra material and, in the future, a DVD which can be purchased at minimal costs from http://www.fungalcell.org.

110. Possible role of structural componenets of the secretory pathway in the replication cycle of*Cryphonectria parasitica*. Massimo Turina, Debora Wilk, and Neal Van Alfen. UC-Davis, Department of Plant Pathology, Davis, CA.

CHV1 infection of the filamentous ascomycete *Cryphonectria parasitica*, the causal agent of chestnut blight, results in hypovirulent phenotype. Previous studies showed a consistent proliferation of host vesicles where virus replication and dsRNA accumulation occur. Heavy water-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 whereas in healthy mycelia the amount of vesicles dramatically decreases after an early accumulation. A subset of vesicles shown to contain viral dsRNA and proteins reacting to CHV1 helicase and polymerase antibodies appeared to be coated. Moreover the vesicle fraction of CHV1 infected *C. parasitica* contain an enriched protein band reacting with anti-clathrin heavy chain antibodies and anti- middle component of its adapter complex 1 in western blot analysis. The finding that clathrin coat associated protein accumulates in hypovirulent strains of *C. parasitica* prompted us to clone the C. parasitica clathrin heavy chain gene and the middle component of its adapter complex 1 in order to investigate their role in CHV1- *C. parasitica* interaction.

111. Intracellular processing and secretion of the fungal hydrophobin cryparin. Massimo Turina, Pam Kazmierczak, and Neal Van Alfen. Department of Plant Pathology, University of California, Davis, California.

Cryparin is an abundant class II fungal hydrophobin found in the cell walls of fruiting bodies of the fungus *Cryphonectria parasitica*. This protein is necessary for the eruption of the fungal fruiting body through the bark of infected trees. Large amounts of cryparin are secreted in liquid culture allowing its use to study vesicular protein secretion. The preprocryparin is processed by cleavage of the signal peptide and then the propeptide is cleaved by a Kex2-type of endoprotease. The role of the Kex2-type processing on secretion of this protein was studied by site-specific mutagenesis of the Kex2 recognition site. Antibodies were raised against a His-tag cryparin fusion protein and used in Western blot analysis of sub-cellular fractions and culture fluid of C. parasitica. GFP fusion was also used to study the secretion of this protein. Results indicate that Kex2 processing is not necessary for secretion and that coated vesicles are involved in the secretion of cryparin.

Developmental Biology

112. Pheromone discrimination in *Schizophyllum commune* and evolutionary aspects. Susanne Gola and Erika Kothe. Friedrich-Schiller-University, Institut für Mikrobiologie, Mikrobielle Phytopathologie, Winzerlaer Str. 10, 07745 Jena

The tetrapolar mating type is defined in part by a multi-specific pheromone/receptor system. The interaction of pheromone receptors and their non-self ligands, encoded by the *B* loci of *Schizophyllum commune*, lead to sexual development. There is no induction of sexual development by pheromones of self specificity, of which several are encoded within the locus. Using chimeric receptors composed of *Balpha1* and *Balpha2* specificity new phenotypes were found. Single point mutations allowed to further characterize the interactions between ligands and receptors *in vivo*. Our results are in accordance with a multi-state receptor activation for this G protein coupled receptor. The evolution of multiple, independent and multispecific pheromone receptors and several pheromones encoded in the mating type loci is a feature unique to homobasidiomycete fungi. A model for the evolution of new specificities by recombination and selection is proposed which explains evolution of new specificities. The model makes use of the multi-state receptor activation explaining differential responses to different pheromones in one receptor molecule.

113. A yeast-like pheromone/receptor system is involved in the sexual development of mycelial ascomycetes. Stefanie Poeggeler and Ulrich Kuck. Department of Botany, Rhur-University Bochum, Germany

In order to analyze the involvement of pheromones in cell recognition and mating in homothallic and heterothallic ascomycetes, two putative pheromone precursor genes, named *ppg1* and *ppg2*, were isolated from the homothallic fungus *Sordaria macrospora*. The *ppg1* gene is predicted to encode a precursor 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. Disruption of both pheromone precursor genes in *S. macrospora* revealed that pheromones are involved in the sexual development of a homothallic fungus. Detection of pheromone genes in filamentous ascomycetes implicated the presence of pheromone receptor genes. We have identified two pheromone receptor genes, named *pre1* and *pre2*, in each of the genomes *Neurospora crassa* and of *S. macrospora*. The deduced *pre1* gene products are putative seven-transmembrane proteins, which display a high-level amino acid identity with the *S. cerevisiae* a-factor receptor Ste3p, and are also homologous to lipopeptide pheromone receptors of basidiomycetes. The deduced *pre2* products display significant sequence similarity with the yeast *STE2* gene product, the alpha-factor receptor. The two genes are transcriptionally expressed in both *N. crassa* and *S. macrospora*. Northern and RT-PCR analyses indicate that in *N. crassa*, expression of the receptor genes does not occur in a mating type specific manner.

114. SwoHp, A Nucleoside Diphosphate Kinase, Is Essential in *Aspergillus nidulans.* Xiaorong Lin[1], Cory Momany[2], and Michelle Momany[1], [1]Plant Biology, [2]Pharmaceutical and Biomedical Sciences, University of Georgia

The ts- *swoH-1* mutant of *A. nidulans* was swollen and lysed during vegetative growth at restrictive temperature. The *swoH* gene was mapped to chromosome II and cloned by complementation. The sequence showed that the *swoH* gene encodes a homologue of nucleoside diphosphate kinases (NDKs). Cell extract from the *swoH-1*mutant is low in NDK activity. Sequence of the mutant allele and structural modeling suggested that the *swoH-1* mutation distorts the enzyme active site. The *swoH* gene fused with 3xHA tag at the N-terminus complemented *swoH-1* mutant indicating the functional integrity of the fusion protein. The tagged protein possessed the phosphate transferase activity as analyzed by TLC. Similar to other eukaryotes, SwoHp in *A. nidulans* forms a hexamer based on results of western blot of a native gel and subunit interactions of the modeled protein structure. Although NDK has been considered a housekeeping enzyme in nucleotide metabolism, recent evidence suggests that it is also an important regulatory protein in many cellular processes. In mammals, NDKs are involved in differentiation and tumor metastasis. In plants, NDK interacts with the phytochrome molecules. Although null mutants of *E. coli, S. cerevisiae* and *S. pombe* are viable, deletion of the *swoH* gene was lethal in *A. nidulans* suggesting that NDK plays a more important role in this filamentous fungus.

115. Heterologous microarray hybridization identifies genes regulated differentially during sexual development of *Sordaria macrospora.* Minou Nowrousian, Stefanie Pöggeler, Carol Ringelberg, Jennifer J. Loros, Jay C. Dunlap, Ulrich Kück. Ruhr-University Bochum and Dartmouth Medical School.

The filamentous fungus *Sordaria macrospora* develops complex fruiting bodies (perithecia) to propagate its sexual spores. Not much is known about gene expression patterns during this developmental process; therefore, we have used microarray hybridization to identify genes that are differentially regulated in the wild type compared to the developmental mutant *pro1*. We have made use of the fact that *S. macrospora* is a close relative of *Neurospora crassa*, for which microarrays have been established previously. Microarrays spotted with *N. crassa* probes were hybridized with targets derived from *S. macrospora* RNA. Using this heterologous array hybridization, we were able to identify a number of candidate genes that are more than threefold up- or downregulated in the *S. macrospora* developmental mutant *pro1* compared to the wild type. For several of these genes, verification experiments were performed using Northern blots or real time PCR, and in all cases confirmed the expression patterns observed on the arrays. As the mutant *pro1* lacks the zinc finger protein PRO1 which is a putative transcription factor, at least some of the genes that are transcriptionally up- or downregulated in the mutant strain might be target genes directly regulated by the PRO1 protein. Among the genes that are upregulated in the mutant strain is *ppg1* (*pheromone precursor gene 1*), which in *S. macrospora* and other fungi has been shown to be involved in sexual development.

116. A protein kinase of *Phytophthora infestans* induced during zoosporogenesis has a novel structure. Flavio A. Blanco, Samuel Roberts and Howard S. Judelson. University of California, Riverside, CA, USA.

Zoosporogenesis in *P. infestans* is a critical step in infecting plants. Proteins involved in this process are good targets for disease control. A gene expressed during zoospore formation was identified that showed high similarity to protein kinases. The predicted sequence of the protein contains all 12 domains diagnostic of Ser/Thr kinases, and the best similarity to the Ca^{+2}/CaM family. However, it lacked the long C terminal regulatory domain typical of such proteins, while having a longer N terminus. The promoter of this gene was cloned upstream of the *gus* gene and the construct was used to transform *P. infestans*.*Cis* elements involved in expression during zoosporogenesis will be discovered by deletion analysis and associated transcription factors will be identified through one-hybrid screening. To study the activity of the protein, it was expressed as a fusion with the maltose binding protein in *E. coli*. Antibodies were raised to study the accumulation of the kinase during life cycle, its subcellular localization and interacting proteins. At the same time, the kinase was used as bait in a two-hybrid screening of a cDNA library from different stages of zoosporogenesis (from sporangia to swimming zoospores). Several interactors were identified which may represent substrates and regulatory proteins. These studies will reveal the role and regulation of this kinase and identify other components of signal transduction pathways involved in zoosporogenesis.

117. Negative control of asexual sporulation in *Aspergillus nidulans*: FluG suppressor analyses. Jeong-Ah Seo, Yajun Guan, and Jaehyuk Yu. Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, WI 53706,USA.

Asexual sporulation (conidiation) in *Aspergillus nidulans* requires an early developmental activator called FluG. Loss of *fluG* function results in the blockage of both conidiation and production of the mycotoxin sterigmatocystin. Molecular mechanisms of FluG-mediated activation of conidiation are unknown. To investigate molecular events responding to FluG, we carried out an unbiased genetic analysis and have isolated and characterized 40 suppressor mutants that overcome the sporulation defects of a loss of *fluG* function mutant (suppressor of FluG; SFG). Genetic analyses revealed that an individual suppression is the result of a single second site mutation not linked to *fluG*, and that most *sfg* mutations are recessive, only one is dominant. Among many recessive SFGs, we have identified two allelic suppressors (*sfgA*) that are predicted to encode a novel Zn2Cys6 DNA binding protein of which mRNA is differentially expressed during the lifecycle. Miss-sense mutations in SfgA defined by these allelic suppressors are expected to cause a loss-of-function suggesting that SfgA negatively regulates conidiation downstream of *fluG*. Moreover, complete loss of *sfgA* not only suppresses loss of *fluG* functions, but also induces conidiophore formation in liquid submerged culture within 22 hrs indicating that FluG's main role in activation of conidiation is to remove the negative effects imposed by SfgA. Molecular genetic analysis leading to a finding of the involvement of at least two loci in suppression of *fluG* is also presented.

118. Polar growth in *Aspergillus nidulans* requires protein modification: an examination of *swoA* and *swoF*. B. D. Shaw, and M. Momany. Department of Plant Biology, Plant Science Building, University of Georgia, Athens, GA, 30602, USA.

Two swollen cell (*swo*) temp. sensitive *A. nidulans* mutants that are aberrant in polar growth were previously complemented and their corresponding genes were cloned and sequenced. SwoA is a protein mannosyl transferase responsible for the first step in protein O-glycosylation, i.e. the co-translational addition of a mannose residue to serine or threonine residues in the endoplasmic reticulum . The *swoA* mutant grows isotropically producing giant >50 micrometer diameter round cells that do not send out a polarized germ tube. SwoF is an N-myristoyl transferase responsible for attaching a 14 carbon fatty acid, myristate, to the N-terminus of a small subset of proteins. This modification is thought to increase the affinity of the target for hydrophobic membranes. The *swoF* mutant can send out a polarized germ tube but with 10 micrometers of growth the tip ceases extension and begins to swell. Strategies to identify the role of these protein modifications in polar growth will be discussed, including a 2D proteomics approach comparing total protein from wild type and mutant cells. Progress in mutant screens to identify additional swollen cell mutants will be discussed. To date at least six new single locus *swo* mutants have been identified.

119. The *Noxa* Gene Encodes a Gp91phox Homologue Necessary for Sexual Development in *Aspergillus nidulans*. Lara-Ortiz, T. and Jesús Aguirre. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, 04150 México, D.F.

gp91phox is the catalytic subunit of the respiratory burst oxidase, an NADPH dependent, superoxide generating oxidase from phagocytes. Recently, a new family of gp91phox homologues (Nox) has been described in non-phagocytic mammalian cells, which have been implicated in growth factor signaling, mitogenic responses, apoptosis and oxygen sensing. Although no Nox enzymes were reported in microorganisms, Biochemical evidence from our laboratory suggested the presence of a NADPH oxidase activity in *A. nidulans*, which led us to request *Cereon Genomics LLC* for possible gp91phox homologue genes. A 720 bp PCR product was amplified using primers based upon provided partial genomic sequence. Cosmids identified with this fragment were used to obtain entire noxA sequence. Predicted NoxA protein is 47% identical to human gp91phox and highly similar to other Nox enzymes. To characterize *noxA* function, a disruption construct was generated replacing part of the *noxA* coding region with selective marker *argB*. *noxA* null mutants were unable to develop sexual fruit bodies, indicating that *noxA* is necessary for sexual development. Accordingly, *noxA* transcript was induced during sexual development and derepressed in *sakA* null mutants, which show premature sexual development. Our results indicate that reactive oxygen species are involved in *A. nidulans* sexual development.

120. Identifying genes involved in asexual sporulation of *Phytophthora infestans* using cDNA macroarrays. Kyoung Su Kim and Howard S. Judelson, University of California, Riverside, CA USA

Asexual sporangia are the main agents of dispersal of *P. infestans*, and the main inoculum for the potato and tomato late blight. To understand the factors involved in differentiating sporangia from hyphae, and to identify spore components that participate in germination, 5200 sequenced clones from a sporangial cDNA library were spotted on membranes and hybridized with total cDNA from sporangia, hyphae, germinated spores, starved hyphae, and sporulation-defective strains. This identified 54 genes up-regulated >5-fold in sporangia. For 43 genes, searches against GENBANK predicted functions in regulation (transcription factors, protein phosphatases and kinases, signal transduction, etc.), structure, transport, and metabolism. The most common type of gene induced in spores (>10% of mRNA) were polyol dehydrogenases. These plus selected others were also induced during starvation. The genes could also be grouped based on whether they continued to be expressed at high levels after germination, or in sporulation-defective strains. This identified genes that may participate in early versus later stages of spore biology. The cellular roles of genes with regulatory functions, such as transcriptional activators, are currently being characterized by gene silencing, yeast two-hybrid, and other methods. Fusions between GUS and spore-induced promoters are also being dissected to identify the mechanisms by which the genes are activated.

121. The COP9 signalosome is an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. S. Busch, S. E. Eckert (1), S. Krappmann, G. H. Braus. Institute of Microbiology and Genetics, Department Molecular Microbiology and Genetics, Georg-August-University, Grisebachstr. 8, D-37077 Goettingen,

Germany. (1) Present address: Department of Biosciences, Faculty of Science, Technology and Medical Studies, University of Kent at Canterbury, Canterbury, Kent CT2 7NJ, UK.

We identified the COP9 signalosome (CSN) as an essential regulator of development in the filamentous fungus *Aspergillus nidulans*. The deduced amino acid sequences of the two genes *csnD* and *csnE* show up to 38% and 53% identities to the fourth and fifth CSN subunits of higher eukaryotes. The *A. nidulans* CSND protein is accumulated in the nucleus and interacts with CSNE in a yeast two-hybrid assay. Deletion of either *csn* locus resulted in viable strains with identical developmental phenotypes which could be complemented by the according wild-type genes. When grown in an air-medium interface, vegetative cells of *A. nidulans csn* deletion strains were impaired in cell polarity and showed changes in secondary metabolism resulting in the accumulation of a red pigment. The *csn* deletion strains showed disturbed light-dependence of developmental initiation processes, including constitutive repression of the onset of the asexual reproductive cycle in developmentally synchronised and competent mycelia. The *deltacsn* mutants were capable to initiate the sexual cycle and develop primordia of fruit bodies. However, the further maturation and morphogenesis of primordia to sexual fruit bodies was blocked. This developmental arrest could not be overcome by overexpression of the sexual activator velvet (VEA). We conclude that the COP9 signalosome is a key regulator in *A. nidulans* which is essential for light-dependent signalling and sexual development.

122. Characterization of differentially expressed genes in dimorphism and pathogenesis in *Ustilago maydis*. María D. García-Pedrajas, David L. Andrews and Scott E. Gold. Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274

Morphogenesis and pathogenesis are closely related in the corn pathogen *Ustilago maydis*. Upon mating of compatible haploid sporidia *U. maydis* switches from budding growth to a filamentous dikaryon. This transition in morphology coincides with a change from saprophytic to pathogenic development. Production of teliospores, the only cells competent to undergo meiosis, occurs only inside the plant, therefore the fungus is completely dependant on the plant to complete its life cycle. 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 a number of genes upregulated during filamentous or budding growth and confirmed differential expression by northern blot analysis. We have produced disruption mutants of select genes to determine their biological roles 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. We have constructed two libraries enriched for genes up and downregulated during gall formation, respectively. Screening of these libraries by reverse northerns has revealed many genes differentially expressed in galls versus infected non-galled tissue. We are now in the process of characterizing individual genes from both libraries.

123. Analysis of the Spore Killer Phenotype of the Ascomycete *Podospora anserina*. Andrea Hamann, Jens Rabenstein, Heinz D. Osiewacz. J. W. Goethe-Universitaet, Botanisches Institut, Frankfurt/Main, Germany

In some filamentous fungi a special form of meiotic drive, spore killing, can be observed. Crosses between a so-called killer strain and a sensitive strain result in the elimination of spores carrying exclusively the sensitive allele. The ascomycete *Podospora anserina* normally produces four heterokaryotic – linearily ordered – ascospores. In case of spore killing, only those spores survive that carry at least one killer allele. Spores exclusively containing sensitive alleles degenerate early in development. The nature of the corresponding alleles and the cause for the degeneration of the sensitive spores remain speculative. The degeneration may be triggered by the strictly regulated transposition of mobile elements or by epigenetic factors. To identify components involved in spore killing, two different strategies were followed. Both strategies employ a subtractive suppressive hybridization technique (SSH). First, the genome differences between a killer and a sensitive strain are analysed. Second, genes are identified which become differentially expressed during sexual development and especially during spore killing. Northern and sequence analysis of these genes are presented and discussed.

124. Isolation of the *bad42* gene of *Coprinus cinereus*. Patricia J. Pukkila, Paul Medina, and Suzanne Harrold Dept. Biology U. North Carolina-Chapel Hill

We have taken advantage of several features of the *C. cinereus* mating system to recover many recessive mutations (induced by UV) that block basidiospore development (bad mutants). To facilitate molecular analysis of these genes, we have identified new *bad*alleles generated by transgene insertions that fail to complement the original mutations. We have used TAIL PCR to obtain sequences flanking the transgene insertions, and recovered cosmid clones that complement the meiotic defects for *bad42*. Sequencing of a cosmid clone, cDNA clones, and PCR products from the UV-induced allele revealed that the original mutation is a C to T transition generating an in-frame stop codon 392AA upstream from the 3' end of the gene. The non-complementing REMI mutation is a rearranged transgene inserted 215AA upstream from the 3' end. The *bad42* gene encodes a basic protein with limited similarities to other gene products that have been previously implicated in meiosis. In *C. cinereus, bad42* is a typical class II mutant which exhibits full length axial cores with very little SC. Both the point mutant and the insertion mutant exhibit metaphase I arrest. The cosmid clone integrated at random genomic positions complements the *bad42* mutation both when homozygous and when heterozygous. We conclude that either "meiotic silencing by unpaired DNA" does not occur in *C. cinereus*, or that *bad42* sequences escape MSUD, either because of the timing of their expression or because of the particular genomic locations of these ectopic cosmid insertions.

125. External pH Affect Secretion of Pectate Lyase by *C. gloeosporioides*. H. Kramer- Haimovich[1] , N. Drori[1] , J. Rollins[4], Y. Okon[2], O. Pines[3] and D. Prusky[1]. [1]Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel. [2]Department of Plant Pathology and Microbiology, Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences, Rehovot 76100, Israel, [3]Department of Molecular Biology, Hebrew University, Medical School, Jerusalem 91120, Israel. [4]Department of Plant Pathology, University of Florida, Gainesville, Florida 32611, USA.

Accumulation of ammonia and associated tissue alkalinization predisposes fruit to attack by *C. gloeosporioides.* As external pH increases from 4.0 to 6.0, pectate lyase (PL) and other extracellular proteins are secreted and accumulate. At pH 4.0 neither *pelB* (encoding PL) transcription nor PL secretion were detected; however they were detected as the pH increased. Sequence analysis of the 5' upstream region of the *pelB* promoter revealed nine putative consensus-binding sites for the *Aspergillus* transcription factor PacC. Accordingly transcript levels of *pac1* (the *C. gloeosporioides pacC* homolog) and *pelB* increased in parallel as a function of pH. Our results suggest that ambient pH is an independent regulatory factors for processes linked to PL secretion and virulence of *C. gloeosporioides*.

126. Factors Controlling Expression of Mep2p in *Saccharomyces cerevisiae*: interplay of phosphorylation state and the cAMP-dependent PKA pathway. Casey Shawler, David Smith, Michael H. Perlin. University of Louisville, Louisville, Kentucky, USA

The Mep2 protein acts as both a high-affinity transporter of ammonium and as a sensor tied to a signal transduction pathway that, under conditions of nitrogen limitation, shifts budding yeast cells into psuedohyphal growth. Genetic evidence ties Mep2p to the cAMP-dependent PKA pathway, since *mep2* deletion mutants defective in the pseudohyphal response are corrected by addition of exogenous cAMP or by bypass of cyclase mutations (*bcy*) that allow constitutive PKA. Interestingly, we have identified in Mep2p (see poster by Smith, Shawler, Lovely, and Perlin) a putative target for phosphorylation by PKA. We have made several site-directed mutants in this position, one of which mimicks phosphorylation. In order to examine possible interactions that regulate Mep2p expression, we have constructed fusions between the *MEP2* promoter and the *lacZ* gene. This construct was placed into wildtype *S. cerevisiae* as well as strains with mutations in different genes within the PKA pathway. Moreover, expression was also examined for strains containing a second construct in which the Mep2p produced contained a mutation that made the protein appear phosphorylated. Like the wildtype protein, this mutant was able to complement the pseudohyphal defect in a strain deleted for the three *MEP* genes. However, expression of the mutant Mep2p in a*tpk2/tpk2* deletion strain, defective in PKA activity, allowed this strain to overcome its pseudohyphal defect on low ammonium, suggesting that over-expression of the "modified" Mep2p is epistatic to PKA. When these results are combined with those on transcriptional effects of mutations in the PKA pathway, an interesting picture will begin to emerge of the relationships between this conserved ammonium transporter, the even more highly-conserved PKA pathway, and their respective roles in dimorphism of fungi.

127. Role of inositol triphosphate and calcium signalling in gene expression during zoosporogenesis in *Phytophthora infestans*. Shuji Tani, Flavio A. Blanco, and Howard S. Judelson. Department of Plant Pathology, University of California, Riverside, California 92521, USA.

Most infections by the oomycete *Phytophthora infestans*, the late blight pathogen, are initiated by zoospores released from asexual sporangia. This release occurs at cool temperatures in a process known to involve Ca^{2+} or other cations. At higher temperatures, germ tubes emerge directly from sporangia. To better understand these pathways, we tested the effects of inhibitors on the release of zoospores, direct germination, and gene expression during zoosporogenesis. Inhibitors of ion channels, kinases, and inositol triphosphate (IP_3) pathways impaired zoospore release, but not direct germination. To identify genes induced during zoosporogenesis, candidates from EST database mining approaches were tested by Northern blotting. This identified 12 genes induced in sporangia undergoing cleavage into zoospores, but not in directly germinating sporangia. Most genes were repressed by one or more inhibitors, although a broad spectrum of quantitatively distinct responses were observed and some genes were insensitive to all inhibitors tested. The majority of genes were repressed drastically by 2APB, an inhibitor of IP_3-induced Ca^{2+} release, and by the phospholipase C inhibitor U73122. The functions and transcriptional regulation mechanisms of the cleavage-induced genes are now being examined. Emphasis is being placed on studying a family of transcription factor-binding proteins including the analysis of interactors identified by yeast two-hybrid analysis.

128. The role of the transcriptional regulator RfxA during growth and development of the dimorphic pathogen *Penicillium marneffei*. Hayley Smith, Michael J. Hynes and Alex Andrianopoulos. Department of Genetics, The University of Melbourne, Parkville 3010, Australia.

Penicillium marneffei is a thermally dimorphic ascomycete capable of causing infection in immunocompromised individuals. At 25℃, *P. marneffei* exhibits filamentous growth and is also capable of undergoing asexual development to produce uninucleate conidia. Upon transfer to 37℃ a process known as arthroconidiation occurs where cellular and nuclear division become coupled, double septae are deposited and fragmentation of the hyphal filaments leads to the production of uninucleate yeast cells that divide by fission. We are interested in examining the regulatory networks controlling cell-type specificity, development and pathogenesis in *P. marneffei*. The RFX proteins (Regulatory Factor X) comprise a family of transcriptional regulators containing a novel DNA binding domain, the winged helix motif, which is highly conserved from humans to yeast. Genes encoding RFX-type proteins have been isolated from a number of fungi. In *S. cerevisiae* Crt1p is required for regulating the expression of DNA damage-inducible genes, while the essential sak1 protein of *S. pombe*functions downstream of PKA and is required for the completion of mitosis and entry into either stationary phase or the sexual differentiation pathway. The *cpcR1* gene of *A. chrysogenum*, initially identified as a key regulator of cephalosporin C biosynthetic genes, is also required for the hyphal fragmentation of mycelium in liquid culture leading to the production of short, swollen yeast-like arthrospores. Given that this process is highly analogous to arthroconidiation in *P. marneffei*, common mechanisms may be acting to regulate this morphological transition. A *P. marneffei cpcR1* homologue, *rfxA*, has been cloned using degenerate PCR and displays significant homology to other fungal RFX family members. The role of *rfxA* during development and yeast-hyphal morphogenesis is being investigated through the analysis of both *rfxA* deletion and overexpression mutants.

129. Development, oxidative and general stress signal transduction in *Aspergillus nidulans*. Laura Kawasaki, Olivia Sanchez, Kasuhiro Shiozaki[2], Teresa Lara-Ortiz and Jesus Aguirre, Instituto de Fisiolgia Celular-UNAM, Apartado Postal 70-242, 04510 Mexico, D.F. and [2]University of California-Davis.

We have proposed that reactive oxygen species play a central role in microbial development. During the past years, our group has characterized several catalase genes and studied their regulation, as an effort to understand the antioxidant response and its relation to development in *Aspergillus nidulans*. Recently, we have found that the *sakA* gene encodes a member of the Hog1/Spc1/p38 stress MAPK family. SakA MAPK is activated in response to oxidative and osmotic stress and mediates part of catalase gene regulation. On the other hand, a*sakA* null mutant shows development and cell-specific phenotypes. First, it displays premature *steA*-dependent sexual development. Second, it produces asexual spores that are highly sensitive to oxidative and heat shock stress and lose viability upon storage. Indeed, SakA is transiently activated early after induction of conidiation. Our results indicate that SakA

MAPK is involved in stress signal transduction, catalase gene regulation, repression of sexual development, and is required for spore stress resistance and survival. At least part of the effects on sexual development is related to regulation of the NADPH oxidase ecoding gene *noxA*, which is essential for this process. We thank CONACyT and PAPIIT-UNAM, Mexico for financial Support and Cereon Genomics LLC for partial *noxA* DNA sequence.

130. PiCDC14 expression is specific to asexual sporulation in the oomycete *Phytophthora infestans.* Audrey M V Ah Fong and Howard S Judelson. Department of Plant Pathology, University of California Riverside, CA 92521.

CDC14 protein phosphatases have been shown to control mitotic exit and/or cytokinesis in a range of ascomycetes and metazoans. Their genes are normally constitutively transcribed and regulated post-translationally. We have identified a CDC14 homologue from the oomycete *P. infestans*, designated PiCDC14, which interestingly was transcribed only during sporulation. The gene was identified using an *in silico* data mining strategy for developmentally regulated phosphatases. The gene is single-copy in Phytophthora and complemented the *S. cerevisiae cdc14-ts* mutant. Northern blot and promoter::GUS fusion analyses showed that PiCDC14 is only expressed during asexual sporulation, and not in non-sporulating hyphae or cultures forming sexual spores. PiCDC14::GUS activity was detected in sporangiophore initials and along the length of sporangiophores bearing immature sporangia, and upon maturation was restricted to sporangia. PiCDC14 mRNA was present in zoospores and cysts, but disappeared from germlings a few hours before the onset of mitosis. The analysis of transformants from homology-dependent silencing experiments supported the role of PiCDC14 in sporulation. It may synchronize nuclear division during sporulation, and then help maintain sporangia in a state of dormancy by inhibiting cell division and growth. The absence of PiCDC14 during vegetative growth may explain why nuclear division is normally asynchronous in hyphae.

131. Genomic analysis of the transcription factors controlling pseudohyphal growth in *S. cerevisiae*. Anthony R. Borneman and Michael Snyder. Department of Molecular, Cellular and Developmental Biology. Yale University, New Haven, CT, USA.

When exposed to an environment low in nitrogen, diploid cells of the yeast *S. cerevisiae* undergo a transition from an ellipsoidal, unicellular, vegetative state to a pseudohyphal growth form. Pseudohyphae are characterised as being composed of elongated cells which fail to detach from their mother cell. These cells therefore form branching chains of elongated, conjoined cells that radiate out from the colony, presumably aiding in dispersal to areas of higher nitrogen availability. Several transcription factors, including Tec1p, Ste12p, Sok2p and Phd1p, have been shown to affect pseudohyphal growth through the modulation of downstream effector gene expression. While some of these targets, such as the cell wall flocculin, Flo11p, have been identified, it is likely that there are many other genes which remained to be discovered. Chromatin immunoprecipitation microarrays (chIP chip's) were therefore used to elucidate, on a genomic scale, the binding targets for several transcription factors that have been shown to be involved in pseudohyphal growth. This data has therefore allowed for the formulation of a transcriptional network for pseudohyphal growth which encompasses these transcription factors and their downstream genes. This provides the basis for future experiments into the function of previously unidentified/uncharacterised genes in cellular development in *S. cerevisiae*.

132. Oxidative stress and conidiation in Neurospora crassa. Wilhelm Hansberg, Shaday Michán, Fernando Lledías, Adelaida Díaz, Leonardo Peraza, Pablo Rangel. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México

Morphogenetic transitions of *N. crassa* asexual life cycle are responses to a hyperoxidant state. Catalase activity induction and catalase oxidation by singlet oxygen are consequences of this hyperoxidant state. The two large monofunctional catalases (Cat–1 and Cat–3) and catalase-peroxidase (Cat–2) are resistant to molar concentrations of hydrogen peroxide. They are oxidized by singlet oxygen at the heme, without significantly affecting enzyme activity, but are degraded rapidly. Cat–1 is expressed in non-growing cells, such as hyphae in stationary growth and conidia. Cat-1 is accumulated to high levels in conidia. Crystallographic structure of Cat-1 showed an oxidized heme and an inusual covalent bond at the active site. Cat–2 is associated with lysing cells, such as hyphae in late stationary growth, in conidiating substrate mycelium, and base of aerial hyphae. Cat–3 is associated with growing hyphae and is expressed during late exponential and pre-stationary growth. Cat–3 has a signal peptide and is secreted. Light and oxidative stress induces Cat-3. A Cat–3 null mutant strain showed increased protein oxidation

and carotene levels in the dark, indicating oxidative stress. It formed hyphae aggregates and produced six fold the amount of wild type strain aerial hyphae and conidia. Aerial hyphae developed earlier and in higher number per area than wild type. Results support our hypothesis of cell differentiation as response to oxidative stress. Acknowledgements: CONACyT 33148N, DGAPA/UNAM IN225402-2

133. Catalase-peroxidase of presumable bacterial origin. Leonardo Peraza and Wilhelm Hansberg. Instituto de Fisiología Celular, Universidad Nacional Autónoma de México

Neurospora crassa Catalase-2 (Cat-2) was purified and characterized. It is a homodimer of 83.4 kDa subunits. Absorption spectrum and HPLC analysis indicated that its prosthetic group is a two hydroxyl/two carboxylic porphyrin that is different from protoporphyrin IX. The enzyme has catalase and peroxidase activity with similar kinetic constants to other catalase-peroxidases (CP). An internal peptide from the purified enzyme was sequenced and the entire cat-2 cDNA sequence determined. Comparison between cDNA and the genomic sequence (Whitehead Institute/MIT) revealed an ORF with no introns encoding a 753 amino acid CP. Phylogenetic analysis indicates that Cat-2 corresponds to a typical CP that, together with another fungal CP, diverged from the *Burkholderia* (b-proteobacteria) branch. We suggest a bacterial origin for fungal CPs. Cat-2 transcript and activity were found in late stationary-phase mycelia, in growing hyphae treated with heat shock or H2O2 or growing on fatty acids. Cat-2 was developmentally regulated: after 30 min induction of conidiation by air exposure of a mycelial mat, cat-2 mRNA accumulated and Cat-2 activity was detected after 4 h when aerial hyphae were formed. Both, mRNA and Cat-2 increase further and were present in aerial hyphae and conidia. In general, Cat-2 was associated with cells undergoing lysis. Acknowledgements: CONACyT 33148N

134. An unusual Cys-Tyr covalent bond present in the crystall structure of *Neurospora crassa* catalase-1. Hansberg, W., Díaz, A., Horjales, E., Rudiño-Piñera, E. and Arreola, R. Instituto de Fisiología Celular and Instituto de Biotecnología, Universidad Nacional Autónoma de México

Catalase-1 (Cat-1) is one of the two large monofunctional catalases from *N. crassa*. Cat-1 is associated with non-growing cells and is accumulated in conidia. Cat-1 heme is modified by singlet oxygen during cell differentiation and under stress conditions. Here we present the crystallographic structure of Cat-1 determined at 1.75 Å resolution. The structure of Cat-1 was determined by molecular replacement using *Escherichia coli*HPII catalase as a starting model. Cat-1 has a C-terminal domain with a flavodoxin topology similar to E. coli HPII and a catalase from *Penicillium vitale*. Cat-1 dimer is formed by mutual insertion of the N-terminal into the loop of the wrapping domain between the Q-related subunits. The heme group, not covalently bound to the protein, is buried inside the Cat-1 tetramer at 20 Å from the molecular surface. Substrate arrives to the active site through a hydrophobic channel filled with water. Cat-1 has protoheme IX (heme b) (57%) and an oxidized heme (heme d) (43%), originated by dihydroxylation at ring D and subsequent formation of a spirolactone with the propionyl group of this ring. *E. coli* HPII and a catalase from *P. vitale* also have heme d. Cat-1 has an unusual covalent bond between the sulfur of Cys 356 and Cb of Tyr 379 that coordinates the FeIII of the heme. The distance of Cys-Tyr bond is 1.9 Å. HPII has a similar covalent bond between the essential Tyr 415 and His 392. This bond could be related to Cat-1 resistance to inactivation by substrate. Acknowledgements: CONACyT 33148N

135. Molecular regulation of sclerotial development and sclerotial-specific gene expression in *Sclerotinia sclerotiorum.* Jeffrey A. Rollins, Wayne M. Jurick II, and Ulla K. Benny. Department of Plant Pathology, University of Florida, Gainesville FL.

Sclerotia play a key role in the life cycle of *Sclerotinia sclerotiorum*. These compact, melanized, multihyphal resting structures serve as long term survival structures that myceliogenically and carpogenetically germinate to propagate the fungus. Functional analysis of the pH-responsive transcription factor Pac1 has revealed a key role for Pac1 in sclerotial development and maturation. *pac1* transcript accumulation is low in vegetative hyphae but dramatically elevated during sclerotial development. *pac1* null mutants produced by gene-replacement form coalescing, multihyphal structures without distinct melanized rind layers. Other genes with sclerotial-specific transcript accumulation have been identified from a small EST collection. Amino acid sequence data obtained from tryptic fragments of a major sclerotial-specific storage protein (Ssp) were used to identify a cDNA clone encoding the Ssp protein. *ssp* transcript accumulation is high and tightly restricted to the sclerotium. A second unrelated gene with no known function exhibits an identical pattern of transcript accumulation. The most highly represented clone (ca. 6.5%

of the total) in the EST collection, encodes a profilin homolog. Transcripts of this gene are abundant during vegetative growth, apothecial development, and sclerotial initiation but greatly reduced during sclerotial development and exposure to alkaline pH. The functions of these genes in sclerotial development and the role of Pac1 in their regulation are currently being evaluated.

136. To "P" or not to "P"?:The action of methylammonium permeases in fungal dimorphism depends on their ability to be phosphorylated. David Smith[1], Casey Shawler[1], C. Ben Lovely[1], Joe Heitman[2], and Michael H. Perlin[1]. [1] University of Louisville, Louisville, Kentucky, USA and [2] Duke University, Durham, North Carolina, USA

While first cloned from yeast and Arabidopsis, the genes for methyalammonium permease homologues (MEPs) are found in organisms ranging from bacteria to humans. In*Saccharomyces cerevisiae*, one of the three MEPs, Mep2p, seems to be a nitrogen sensor and plays a direct role in the switch from yeast cells to pseudohyphal growth in this organism. In*Ustilago maydis*, the causative agent of galls on maize, the corresponding homologue, Ump2, is required for the ability of haploid yeast-like cells to grow filamentously on low nitrogen. Moreover, Ump2 can substitute for Mep2p in *S. cerevisiae mep1 mep2 mep3*mutants normally incapable of pseudohyphal growth on low nitrogen. The effects of disruption of*mep2* could be reversed by supplying exogenous cAMP. Such results suggest a connection to the respective cAMP-dependent PKA pathways in these two fungi. Comparison of the amino acid sequences of various MEPs revealed a putative phosphorylation site in MEPs of fungi that was absent in MEPs of other organisms. Site-directed mutagenesis of this site in*mep2* and *ump2*, revealed that replacement of the putative target residue(s) had no effect on growth of *S. cerevisiae* on low ammonium, but eliminated filamentation under these conditions. In *U. maydis*, this mutant still provided filamentation on low ammonium. But, a second mutant in which the amino acid change mimicked phosphorylation failed to complement the filamentation defect of *ump2* knock-outs. Together these results provide a model in which the potential phosphorylation state of MEPs controls their roles in filamentous growth for two disparate fungi.

137. Filamentous growth and ammonium uptake in *Ustilago maydis* and*Saccharomyces cerevisiae*: the methylammonium permease, Ump2, performs both functions. David G. Smith[1], Maria D. Garcia-Pedrajas[2], Scott E. Gold[2], and Michael H. Perlin[1]. [1] University of Louisville, Louisville, Kentucky, USA and [2] University of Georgia, Athens, Georgia, USA

Nutrient sensing plays important roles in fungal development in general, and specifically in critical aspects of pathogenicity and virulence. Many dimorphic pathogens must switch from a yeast-like to a filamentous form in order to cause disease. This switch is also required for the phytopathogenic smut fungi, *U. maydis* and *Microbotryum violaceum*. Three genes encoding methylammonium permeases were identified from these fungi and all the encoded proteins were most similar to Mep2p, the high-affinity permease from *S. cerevisiae* that plays a direct role in pseudohyphal or filamentous growth for that organism. Each of the smut genes was expressed in diploid *S. cerevisiae* mutants deleted for all three *mep* genes (*mep1mep2mep3*). Each could complement the severe growth defect of the *S. cerevisiae* mutant on low ammonium. Moreover, the *U. maydis ump2* gene was also able to complement the pseudohyphal defect characteristic of the mutant yeast. This is the first report of a heterologous *mep* gene capable of restoring pseudohyphal growth in yeast. In *U. maydis*, disruption of *ump2* eliminated the filamentous phenotype of haploid cells on low ammonium. The *ump1ump2* double mutant produced a branched budding phenotype and when grown in liquid medium, appeared to flocculate. Of particular note was that the double mutant regained the ability to filament on low nitrogen; however, the filaments of the double mutant appeared more entangled than wildtype. Although all three types of mutants showed reduced uptake of methylamine, the most significant drop in uptake was seen for the *ump2* mutant and the *ump1ump2* double mutants. These findings allow us to present a model of how ammonium transporters play a role in regulating dimorphic growth in fungi.

138. Switching fungal reproductive mode by manipulation of mating-type gene. Jungkwan Lee[1], Theresa Lee[1], Yin-Won Lee[1], Sung-Hwan Yun[2], and B. Gillian Turgeon[3].[1]School of Agricultural Biotechnology and Research Center for New Bio-materials in Agriculture, Seoul National University, Suwon 441-744, Korea. [2]Division of Life Sciences, Soonchunhyang University, Asan 336-745, Korea. [3]Department of Plant Pathology, Cornell University, 334 Plant Science Building, Ithaca, NY 14853.

Fungi capable of sexual reproduction use heterothallic (self-sterile) or homothallic (self-fertile) mating strategies. In most ascomycetes, a single mating type locus, *MAT*, with two alternate forms (*MAT1-1* and *MAT1-2*) called idiomorphs, controls mating ability. In heterothallic ascomycetes these alternate idiomorphs reside in different nuclei. In contrast, most homothallic ascomycetes carry both *MAT1-1* and *MAT1-2* in a single nucleus, usually closely linked. An economically important example of the latter is*Gibberella zeae*, a devastating cereal pathogen of ubiquitous geographic distribution and also a producer of deadly mycotoxins that threaten human and animal health. Because analysis of traits of interest is difficult in homothallic species, we asked if *G. zeae* could be made strictly heterothallic by manipulation of *MAT*. Targeted gene replacement was used to differentially delete *MAT1-1* or *MAT1-2* from a wild type*MAT1-1/MAT1-2* strain, resulting in *MAT1-1/MAT1-0*,*MAT1-0/MAT1-2* strains that were self-sterile, yet able to cross to wild type testers and more importantly, to each other. These results indicate that differential deletion of *MAT*idiomorphs eliminates selfing ability of *G. zeae*, but ability to outcross is retained. They also indicated that both *MAT* idiomorphs are required for self fertility. Furthermore, to our knowledge, this is the first report of complete conversion of fungal reproductive strategy from homothallic to heterothallic by targeted manipulation of *MAT*. Practically, this approach opens the door to simple and efficient procedures for obtaining sexual recombinants of*G. zeae* that will be useful for genetic analyses of pathogenicity and other traits, such as production of threatening mycotoxins.

139. NpgAp encoding the homolog of 4'-phosphopantetheinyl transferase is required for cellular growth and conidiation in *Aspergillus nidulans.* Jung-Mi Kim, Dong-Min Han[1], Keon-Sang Chae, Hwan-Gyu Kim and Kwang-Yeop Jahng. Division of Biological Sciences, Chonbuk National University, Jeonju, South Korea; [1]Division of Life Sciences, Wonkwang University, Iksan, South Korea

In *Aspergillus nidulans*, the *npgA* gene has been known that it plays an important role in pigmentation. We previously isolated and sequenced the DNA fragment that complemented *npgA1* mutation from genomic cosmid library of *A. nidulans*. The *npgA* gene encoded a putative protein of 344 amino acids that has 42% of similarity with phosphopantetheinyl transferase encoded by the *LYS5* gene of *Saccharomyces cerevisae*, which transfers the 4'-phosphopantetheinyl (P-pant) moiety of coenzyme A. The cDNA of *npgA* of *A. nidulans* recover the growth defect of the *lys5* strain of *S. cerevisae*, indicating that *npgA* could functionally complement *lys5* deletion of *S. cerevisae*. The deletion mutant of *npgA* neither grew nor produced secondary metabolites such as the sterigmatocystin and the siderophore. The defect of growth and conidiation in *npgA* knock-out mutant was cured when provided with the culture filtrate of wild type. These results suggested that the NpgAp might be involved in the posttranslational modification of enzymes required for primary growth or the synthesis of secondary metabolites such as pigment and antibiotics in *A. nidulans*.

140. Altering sexual reproductive mode by interspecific exchange of *MAT*loci. Shun-Wen Lu[1], Sung-Hwan Yun[2], Theresa Lee[3] and B. Gillian Turgeon[1]. [1]Department of Plant Pathology, Cornell University, Ithaca, NY 14853 [2]Division of Life Science, Soonchunhyang University, Asan 336-745, Korea [3]School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

Sexual fungi can be self-sterile (heterothallic, requiring genetically distinct partners) or self-fertile (homothallic, requiring no partner). Our goal is to understand the molecular basis for these distinct reproductive modes. The model fungi we have chosen are two closely related filamentous ascomycetes, *Cochliobolus heterostrophus* (heterothallic) and *C. luttrellii* (homothallic). We previously demonstrated that the *C. luttrellii MAT* gene alone conferred homothallism when expressed in a *MAT*-deleted strain of heterothallic*C. heterostrophus*. Here we report a reciprocal study in which the *C. heterostrophus MAT* genes were expressed, separately, in a *MAT*-deleted *C. luttrellii* strain, which is sterile. A *C. luttrellii MAT*-deleted strain carrying Ch*MAT-1* produced fertile pseudothecia when mated with a *C. luttrellii MAT*-deleted strain carrying Ch*MAT-2*. Fertility of pseudothecia was similar to that of wild type. Tetrad analysis confirmed that progeny segregated for parental markers. Surprisingly, each transgenic strain was also able to self although all pseudothecia produced were smaller than those of wild type and the fertility was low (number of asci was about 5% of the number of wild type asci). These data support the argument that the primary determinant of reproductive mode is *MAT* itself.

141. Withdrawn

142. Isolation and characterization of mutants that can sexually develop in the presence of visible light. Jong-Hak Kim, Min-Su Kim, Yoon-Hee Cheon, Keon-Sang Chae[1], Dong-Min Han. Dept., Biol., Wonkwang University, Iksan, Chonbuk 570-749, Korea[1]Div., Biol., Sci., Chonbuk National University, Chonju, Chonbuk, 561-756, Korea

When a homothallic ascomycete, *Aspergillus nidulance*, is exposed to visible light, cleistothecial development is inhibited and instead plenty of asexual spores developed. The ratio of sexual/asexual development increases as light intensity decreases and the sexual development preferentially takes place in dark. This light responsiveness of development implies the existence of delicate regulation process including reception and translocation of light signaling and determination of development. In order to study the genes involved in this regulatory network, we first attempted to isolate mutants that could develop cleistothecia even in the presence of relatively intense visible light. More than 200 mutants were isolated and analyzed. They were divided into two groups. One included those that had defects in the response specific to light and the other those that developed cleistothecia in regardless of the presence of any other inhibitory environmental stress such as high osmolarity. We selected 8 mutants that belonged to the former group. Six mutants were revealed to have single gene mutation and grouped into different complementation groups(*silA-F*). The mutant alleles were all recessive to that of wild type. The *silA* gene was cloned and sequence-analyzed. The putative SilA has a Zn_2Cys_6 zinc finger motif at N terminus and shows high amino acid sequence similarity to Aro80p of *Saccharomyces cerevisiae*.

143. Sexual development in the euascomycete *Podospora anserina*: the role of pheromones. Evelyne Coppin[1], Deborah Bell-Pedersen[2], Dan Ebbole[2] and Robert Debuchy[1]. [1] Institut de Genetique et Microbiologie, UMR8621, 91405 Orsay, France. [2] Texas A&M University, College Station, TX 77843-2132.

The role of pheromones during fertilization has been investigated in several Euascomycetes, but little is known about their function after fertilization. We have cloned the pheromone genes of *P. anserina* to investigate their possible function after fertilization. Degenerate primers have been designed from the alignement of *N. crassa mfa* and *M. grisea MF1-1*and low stringency PCR with these primers allowed us to isolate *mfp*. Low stringency hybridization with *ccg4* of *N. crassa* on *P. anserina* genomic DNA led to the isolation of *mfm*. The deletion of *mfp* results in male sterility in *mat+*strains but does not affect female fertility and has no effect in *mat-* strains. Reciprocally, the deletion of *mfm* results only in male sterility in *mat-* strains. Specific transcription of *mfm* and *mfp* in *mat-* and *mat+* strains indicates that these genes are under the control of the mating-type genes. The genetic analysis of strains containing transcriptionally deregulated pheromone genes suggests that the mating types also control the post-transcriptional events required for the production of active pheromones. Experiments aimed at testing the function of pheromones after fertilization are underway. This work is supported by the ACI Biophys 2001 (project n° 185).

144. Temporal localization of RIP and rearrangements in *Podospora anserina*. Khaled Bouhouche, Sylvie Arnaise and Robert Debuchy. Institut de Génétique et Microbiologie, UMR8621, 91405 Orsay, France.

Genetic analyses in *P. anserina* suggest that RIP and sequence rearrangements (RIP/R) occur between fertilization and premeiotic replication. A few events have been identified during this period in *P. anserina*, offering the possibility to specify the time of RIP/R. Fertilization is followed by the division of male and female nuclei inside a syncytium. The mating-type genes *FPR1*, *FMR1* and *SMR2* control the recognition between male and female nuclei. This step is associated with a developmental arrest which is overcome by the action of *SMR1*. Then pairs of male and female nuclei form dikaryotic hyphae in which premeiotic replication, karyogamy, meiosis and ascospore formation take place. We examined if RIP/R occur before or after the action of each one of the mating-type genes, based on the construction of strains disrupted for each mating-type gene and carrying a cis-duplication of this gene. If RIP/R occur before the action of the duplicated gene, the strain will give a chacteristic progeny indicating that the gene has been altered. If RIP/R occur after the action of the duplicated gene, the strain will not display any phenotype in crosses, but the progeny will produce an affected progeny upon crossing. Preliminary results indicate that RIP/R occur mainly before the action of *SMR1*. Studies are underway to determine if RIP/R occur before or after the action of *FPR1*, *FMR1* and *SMR2*, namely before internuclear recognition or during the developmental arrest.

145. Laccases in the dung fungus *Coprinus cinereus*. M. Navarro-Gonzalez, P. Hoegger, M. Hoffmann, S. Kilaru, R. Dviwedi, M. Zommorodi, A. Majcherczyk, U. Kües. Georg-August-University Göttingen, Institute of Forest Botany, Göttingen, Germany

Laccases (EC 1.10.3.2) belong to the family of multi-copper oxidases and are classified as oxidoreductases acting on polyphenolic substrates. In fungi, they function in lignin and phenol degradation and likely in development and pigment synthesis. Fungal laccases may be secreted into the medium or remain attached to the fungal cell wall. In the basidiomycete *Coprinus cinereus*, laccase activity in the monokaryon can be induced by copper. Moreover, phenol oxidase activity has also been detected when the fungus is grown on wood (oak, poplar) and straw (wheat). *C. cinereus* has at least six different genes for laccases. We cloned these genes from homokaryon AmutBmut and established and compared their sequences. In this strain, being self-compatible due to defects in the mating-type loci, enzymatic laccase function correlates with fruiting body initiation. Inducers of laccases stimulate premature fruiting and enhance initiation frequencies. Laccase activity associates with the mycelium and is not or only poorly detected in the surrounding medium. Work in our laboratory is supported by the DBU (Deutsche Bundesstiftung Umwelt).

146. Mutants in initiation of fruiting body development of the basidiomycete *Coprinus cinereus*. P.-H. Clergeot[1], G. Ruprich-Robert[1], Y. Liu[1], S. Loos[1], P. Srivilai[2], R. Velagapudi[2], S. Goebel[2], M. Künzler[1], M. Aebi[1], U. Kües[2]. [1]ETH Zurich, Institute for Microbiology, Zurich, Switzerland. [2]Georg-August-University Göttingen, Institute of Forest Botany, Göttingen, Germany

Homokaryon AmutBmut is self-compatible due to mutations in the mating-type loci and fruits without the need to mate to another strain. Therefore, we used the strain to produce mutants in fruiting body initiation. Fruiting body initiation can be divided into a dark-dependent step, primary hyphal knot formation, and a light-dependent step, secondary hyphal knot formation. The primary hyphal knot is a loose hyphal mesh that arises on one or a few carrier hyphae through intense localized formation of branches of restricted tip growth. In the dark, it will mature into a sclerotium, a compact globular resting structure with a melanized rind. In contrast, upon light illumination, the loose hyphal mesh of the primary hyphal knot develops by aggregation and further branching into a small mycelial ball of originally uniform structure, the secondary hyphal knot in which stipe and cap tissues of the mushroom will promptly differentiate. In both steps, we identified gene functions by mutant complementation. A new gene in primary hyphal knot formation appears to encode a protein related to *hetE* of*Podospora*, the product of a gene in secondary hyphal knot formation has cyclopropane fatty acid synthase function.

147. The *dst1* gene responsible for a photomorphogenetic mutation in *Coprinus cinereus* encodes a protein with high similarity to WC-1. Katsuyuki Yuki[1], Masashi Akiyama[1], Hajime Muraguchi[2] and Takashi Kamada[1]. [1]Department of Biology, Faculty of Science, Okayama University, Okayama, Japan. [2]Department of Biotechnology, Faculty of Bioresource Sciences, Akita Prefectural University, Akita, Japan.

Light is one of the environmental cues that regulate mushroom (fruit body) development in the basidiomycete *Coprinus cinereus*. When the fungus is grown under a light-dark cycle, normal, fertile fruit bodies develop. However, when the fungus is grown in continuous darkness, ethiolated, slender fruit body primordia, called "dark stipe" form, in which the cap (pileus) remains rudimentary and never develops to produce the fertile fruit body. We isolated and genetically analyzed several blind mutants, which produce "dark stipe" even if proper light conditions are given. From the genetic analysis, we identified two genes, named *dst1* and *dst2*, responsible for the blind phenotype. We then cloned one of the genes, *dst1*, as a DNA fragment that rescues the "dark stipe" phenotype by screening a cosmid library of *C. cinereus*. Sequencing analysis revealed that the*dst1* gene encodes a protein with high similarity to WC-1 of *Neurospora crassa*, which has been proposed to be the blue light photoreceptor.

148. The *eln3* gene responsible for cellular morphogenesis during mushroom development in *Coprinus cinereus* encodes a membrane protein with a general glycosyltransferase domain. Toshihide Arima, Yoshinori Morimoto, and Takashi Kamada. Department of Biology, Faculty of Science, Okayama University, Okayama, Japan.

The hypha grows at the tip during the vegetative phase. However, during mushroom (fruit body) development in homobasidiomycetes, the component hyphal cells of the fruit-body exhibit diffuse extension growth as well as tip

growth to produce a proper shape of the fruit body. In *Coprinus cinereus*, the fruit-body primordium exhibits remarkable expansion during the last phase of development, resulting a slender fruit body with a long stipe (stalk). We isolated a developmental mutant (*eln3-1*) of *C. cinereus*, which produces an aberrant fruit body with a very short stipe, after REMI mutagenesis. Microscopic analysis revealed that the *eln3-1* mutant phenotype is due to the defect in diffuse extension growth of the component cells of the stipe. After plasmid rescue from the genomic DNA of the *eln3-1*mutant strain, we identified the *eln3* gene as a DNA fragment that rescues the *eln3-1* mutation. The *eln3* gene encodes a novel membrane protein of 927 amino acids with seven transmembrane helices and a general glycosyltransferase domain. The level of the *eln3* transcription was much higher in the stipe than in other tissues of the fruit body and in the vegetative hyphae. Also, the level of the *eln3* transcription in the stipe changed in parallel with the elongation rate of the stipe in the course of development.

149. Isolation and characterization of *nsdD* suppressor mutants in *Aspergillus nidulans*. Jee Hyun Kim, Dong Beom Lee, Jung Youl Min, Kap-Hoon Han[1], Kwang-Yeop Jahng[2], Dong-Min Han. Dept. Biol. Wonkwang University, Iksan, Korea, [1]Dept.Food Microbiol.and Toxicol., University of Wisconsin-Madison, Madison,U.S.A, [2]Div. Biol. Science, Chonbuk Univ. Chonju, Korea

The *nsdD* gene encodes a GATA type transcription factor, carrying a type IVb zinc finger DNA binding domain, which functions in activating sexual development of *A. nidulans*. A number of suppressor mutants of *nsdD* were isolated and characterized. According to the phenotypes of growth rate and developmental pattern, nine mutants were selected and recombinants carrying suppressor mutation but free of *nsdD* mutation were isolated through genetic crosses with wild type. Most of suppressor mutants developed plenty of cleistothecia even under the conditions, such as high osmolarity(e.g.1 M KCl), visible light or acetate as a sole C source, where sexual development of wild type is completely inhibited, implying that the genes might be involved in the regulation of sexual development in response to the presence of those environmental factors. All mutant alleles were recessive to that of wild type. They were grouped into five complementation groups and the respective genes were designated as *sndA, sndB, sndC, sndD* and *sndE*. The*sndA* and *sndC* were linked to linkage group II, *sndB* and *sndD* to linkage group V and *sndE* to linkage group VII. Some of those genes including*sndB* were cloned and sequence-analyzed.

150. *veA*-dependent expressions of *indB* and *indD* encoding proteins that interact with NSDD, a GATA-type transcription factor required for sexual development in*Aspergillus nidulans*. Nak-Jung Kwon, Dong-Min Han[1], Suhn-Kee Chae. Research Center for Biomedicinal Resources and Division of Life Science, Paichai University, Daejeon 302-735, Korea and [1]Division of Life Science, Wonkwang University, Iksan 570-749, Korea

In *nsdD* mutants of *Aspergillus nidulans*, no sexual organs including cleistothecia, Hull cells, and primodia were ever found. Molecular cloning of the *nsdD*gene revealed that *nsdD* encoded a putative GATA type transcription factor carrying a type IVb zinc finger motif at the C-terminal end. To clarify the NSDD function, proteins interacting with NSDD have been screened using the yeast two-hybrid system. Two IND (**I**nteractor of **N**SD**D**) proteins, INDB and INDD were isolated and characterized further. Determination of *indB* and *indD* cDNA sequences revealed open reading frames of 648 bp and 642 bp, encoding polypeptides of 215 and 213 amino acids, respectively. INDB and INDD showed 42% amino acid sequence identity and also shared similarities to an ORF in *Neurospora crassa*. NSDD-INDB and NSDD-INDD interactions were confirmed *in vitro* using a GST-pull down assay. Both proteins interacted with the Zn-finger domain of NSDD. The N-terminus of INDB and the C-terminus of INDD were responsible for the NSDD interaction. INDB and INDD was able to interact each other. Self association of INDB but not for INDD was observed, too. In Northern analysis, 1.2 kb transcripts were detected for both genes. The *indB* transcripts expressed to similar levels during asexual development, but increased at 20-30 hour after induction of sexual development, then decreased. In contrast, the *indD* transcript was the most abundant at the initial asexual developmental stage. Furthermore, both kinds of transcripts were highly produced in *veA*mutant strains, but repressed in *veA*$^{+}$ wild type background, indicating*veA*-dependent expressions. *veA* mutations conferred defectiveness in sexual differentiation. Our results suggest that increased expressions of INDB and INDD in *veA*background might affect NSDD function negatively by binding to the Zn-finger region of NSDD to block DNA binding. [Supported by grants from KOSEF]

151. NpgAp encoding the homolog of 4'-phosphopantetheinyl transferase is required for cellular growth and conidiation in *Aspergillus nidulans*. Jung-Mi Kim, Dong Min Han[1], Keon-Sang Chae, Hwan-kyu Kim and Kwang-

Yeop Jahng. Division of Biological Sciences, Chonbuk National University, Chonju, Korea; [1]Division of Life Science, Wonkwang University, Iksan, Korea

In *Aspergillus nidulans*, the *npgA* gene has been known that it plays an important role in pigmentation. We previously isolated and sequenced the DNA fragment that complemented *npgA1* mutation from genomic cosmid library of *A. nidulans*. The*npgA* gene encoded a putative protein of 344 amino acids that has 42% of similarity with phosphopantetheinyl transferase encoded by the *LYS5* gene of *Saccharomyces cerevisae*, which transfers the 4'-phosphopantetheinyl (P-pant) moiety of coenzyme A. The cDNA of *npgA* of *A. nidulans* recover the growth defect of the *lys5*strain of *S. cerevisae*, indicating that *npgA* could functionally complement*lys5* deletion of *S. cerevisae*. The deletion mutant of *npgA* neither grew nor produced secondary metabolites such as the sterigmatocystin and the siderophore. The defect of growth and conidiation in *npgA* knock-out mutant was cured when provided with the culture filtrate of wild type. These results suggested that the NpgAp might be involved in the posttranslational modification of enzymes required for primary growth or the synthesis of secondary metabolites such as pigment and antibiotics in *A. nidulans*.

152. Dissecting the blue light response in *Trichoderma atroviride*. Casas-Flores, S.[1]., Rosales-Saavedra, M., Rios-Momberg, M., Bibbins, M., Ponce-Noyola, P., and Herrera-Estrella, A[2]. Departament of Plant Genetic Engineering, CINVESTAV Unidad Irapuato. Apartado postal 629. 36500., Irapuato, Guanajuato, México.

The biocontrol agent *Trichoderma atroviride* responds to blue light by developing conidia in the colony perimeter where the light pulse was received. Additionally, an increase of the transcripción of *phr1* (photolyase gene) and *tpka1* (protein kinase gene) is normaly detected. In *Neurospora crassa*, *white collar-1* (*wc-1*) and*white collar-2* (*wc-2*) genes are required for blue light response and circadiam rhythms. The corresponding homologous genes to *wc-1* and *wc-2* were cloned in*T. atroviride* (*twc1* and *twc2* respectively). Expression analysis of both*twc1* and *twc2*, showed no significative changes when *Trichoderma*colonies were shifted from dark to light conditions. *twc1* and *twc2* null mutants were generated by gene replacement. Both mutants were unable to produce conidia in response to blue light. Conidiation driven by any other conditions was not affected. Light-induced transcription of *phr1* was completely dependent on functional Twc1 and Twc2. On the other hand, *tpk1* induction was not affected in *twc1*$^{-}$ and*twc2*$^{-}$ strains. Interstingly, both mutants seem to have a differencial behaviour when growing under light or dark conditions These results suggest the presence of at least two different photoreceptors for blue light in *Trichoderma*. Addittionally we are employing an strategie based on a massive molecular analysis of four subtractive cDNA libraries that were obtained during blue light exposure.

153. GanBp, a homolog of G protein alpha subunit, negatively regulates the asexual sporulation in *Aspergillus nidulans*. Mi-Hee Chang[1], Dong-Min Han[2], Keon-Sang Chae[1] and Kwang-Yeop Jahng[1]. [1]Division of Biological Sciences, Chonbuk National University, Chonju; [2]Division of Life Sciences, Wonkwang University, Iksan, Korea.

We isolated *ganA* and *ganB* encoding G alpha protein homologs from*Aspergillus nidulans*. To investigate the cellular function of these G proteins, we have constructed various mutants of *ganA* and *ganB* by gene targeting. The viscosity of culture broth of *ganA* dominant-activating mutant in which GanAp is constitutively activated was lower than that of wild type. The protoplasts were generated more rapidly in this mutant. However deletion, over-expression, or dominant interfering mutants of *ganA*have shown no clear phenotype in growth and development. Meanwhile, *ganB* deletion or dominant-interfering mutants showed hyperactive sporulation phenotype and derepressed*brlA* expression in submerged culture. Constitutive activation of GanBp caused reduction of hyphal growth and asexual sporulation. Cleistothecium formation was accelerated in*ganB* over-expression mutants but reduced in *ganB* deletion and interfering mutants. Consequently, we suggest GanBp probably negative regulator of asexual sporulation resulting in progressing sexual development. In addition, *ganB* deletion or dominant-interfering mutation delayed conidial germination rate and dominant-activating mutation caused precocious germination even without carbon source, implying that GanBp might be involved in carbon source sensing required for conidial germination in *A. nidulans*.

154. Conidiation genes of the plant pathogenic fungus *Fusarium oxysporum*. Toshiaki Ohara, and Takashi Tsuge. Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan.

Fusarium oxysporum is a soil-borne facultative parasite that causes economically important losses on a wide variety of crops. *F. oxysporum* produces three kinds of asexual spores, microconidia, macroconidia, and chlamydospores. Ellipsoidal microconidia and falcate macroconidia are formed from phialides; globose chlamydospores with thick walls are formed acrogenously from hyphae or by the modification of hyphal cells. We identified the *F. oxysporum* homologs of *medA* and *stuA*, which have been identified to encode the developmental regulators for asexual and sexual sporulation in *Aspergillus nidulans*. The *medA* homolog, named *REN1*, was identified by restriction enzyme-mediated integration mutagenesis. The *stuA* homolog, named *FoSTUA*, was isolated by a PCR-based cloning. Although the *REN1* mutants exhibit normal growth and form chlamydospores, they lack microconidia and macroconidia and form rod-shaped, conidium-like cells. Thus, *REN1* is required specifically for development of microconidia and macroconida. The *FoSTUA* mutants produce microconidia, macroconidia, and chlamydospores. Mutation in *FoSTUA*, however, quantitatively affects the development of macroconidia and chlamydospores: numbers of macroconidia and chlamydospores were significantly reduced and increased, respectively, by the mutation. We propose that *REN1* is comprised in the core pathway for development of microconidia and macroconidia in *F. oxysporum*.

155. Roles of a ras homologue in apical growth of Neurospora. Tadako Murayama,Tomomi Edo, and Yasuhiro Ishibashi. College of Engineering, Kanto-Gakuin University, Yokohama 236-8501 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 region where the Nc-ras2 protein functions and roles of the Nc-ras2 protein will be discussed

156. Morphological mutants *of Neurospora crassa*. I. From sequence to phenotype in Neurospora morphogenesis. David D. Perkins, Stanford University.

This is the first of three posters that give different views of mutants with altered morphology (or pigmentation), as seen (1) in living cultures, (2) scanning EM photographs, and (3) conventional micrographs of altered hyphal growth. Living cultures of ~50 representative morphological mutants will be displayed, with primary gene products specified when they are known. Prior to the Neurospora genome project, nearly one-tenth of the predicted 10,000 genes had already been identified and mapped using mutations and classical genetic methods. Many of these mutations were recognized by their effects on morphology. Thirty years ago, E. L. Tatum and others attempted to identify the defects of various morphological mutants and to use them in studies of morphogenesis, but they were frustrated for want of appropriate molecular tools. Molecular identification of primary products of some of the morphologicals was eventually accomplished by other workers, using cloned genes at loci that had previously been defined using mutant phenotypes and classical genetic methods. For example, the wild type allele of*crisp*, one of the first mapped Neurospora mutants, was cloned, sequenced, and shown to specify adenylate cyclase. Now, ESTs and information from the genome project enable us to go in the reverse direction, from DNA sequence to phenotype. Inactivation of genes that were originally defined by molecular sequence reveals that many of them affect morphology. Predicting mutant morphology solely from a priori knowledge of sequence will usually be difficult or impossible. However, it may be possible to rationalize the phenotypic effect once the mutant morphology is known. Unravelling the web of morphogenetic events that lead from primary gene product to visible phenotype remains a daunting challenge.

157. Morphological mutants of *Neurospora crassa*. II. Scanning EM photographs. Matthew L Springer, Stanford University.

This is the second of three posters giving different views of mutants with altered morphology. Examination of morphological mutants by scanning electron microscopy provides information to supplement what is known from gross morphology. The photographs of morphological mutants that are shown here can be accessed via the FGSC web site at http://www.fgsc.net/neurosimages/neuimage.htm or via the American Society for Microbiology web site at http://www.microbelibrary.org/Visual/page1.htm. Methodology used in preparing material for photography is described in Springer and Yanofsky 1989, Genes and Development 3:559-571. Information on the individual mutants, and references to published sources, are given in Perkins, Radford, and Sachs, 2001. *The Neurospora Compendium: Chromosomal Loci* (Academic Press), available on line at http://www.fgsc.net/. Living cultures of all the mutants pictured here can be seen in the preceding poster.

158. Morphological mutants of *Neurospora crassa*. III. Hyphal morphology mutants. Stephan Seiler, University of Munich and Michael Plamann, University of Missouri-Kansas City

This is the third of three posters giving different views of mutants that alter morphology. Cellular polarity is a fundamental property of every cell. To identify the critical components that contribute to polarized growth, we developed a large-scale genetic screen for the isolation of conditional mutants defective in polar and directed growth in the model fungus *Neurospora crassa*. Phenotypic analysis and complementation tests of ca. 950 mutants showing defects in hyphal growth identified more than 100 complementation groups that define 20 distinct morphological classes. The phenotypes range from polarity defects over the whole hypha or more specific defects localized to hyphal tips and subapical regions to defects in branch formation and growth directionality. To convert this mutant collection into meaningful biological information, we identified the defective genes in 45 mutants covering all phenotypic classes. These genes encode novel proteins as well as proteins which (i) regulate the actin and microtubule cytoskeleton, (ii) are kinases or components of signal transduction pathways, (iii) are part of the secretory pathway, and (iv) have functions in cell wall formation or (v) membrane biosynthesis. These mutants highlight the dynamic nature of a fungal hypha and establish a molecular model for hyphal growth and polarity.

159. The homolog of yeast NDT80 is involved in vegetative growth in *Neurospora crassa.* Qijun Xiang and Louise Glass. Dept of Plant&Microbial Biology, University of California, Berkeley, CA94720

The budding yeast protein Ndt80 belongs to a newly defined transcription family. It functions at pachytene of yeast gametogenesis to activate transcription of a large group of genes at the end of meiotic prophase. A putative *Neurospora* protein shares high similarity with Ndt80p. This NDT80 homolog was mutated by RIP (Repeat Induced Point mutation) and a number of mutants have been obtained. The majority of the mutants have shortened aerial hyphae and profuse conidiation pattern, a phenotype similar to *vib-1* mutants that we have reported previously. *vib-1* is involved in mediating vegetative incompatibility. Interestingly, The NDT80 homolog and VIB-1 also share a conserved region with ~100 amino acids. We are examining whether or not the NDT80 homolog is also involved in vegetative incompatibility and meiosis.

160. Further analysis of the cAMP pathway in the control of dimorphic growth in *Ustilago maydis.* Scott E. Gold, John D. Egan*, María D. García-Pedrajas Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274 *Current address, Dept of Biology, Salisbury University, Salisbury, MD

The cyclic AMP pathway is important in the determination of morphological phase in a number of dimorphic fungi. In the corn smut pathogen, *Ustilago maydis*, activation of the cAMP dependent protein kinase (PKA) pathway generates budding growth. Earlier we had identified specific components of the pathway in a forward genetic approach. Here we report results related to reverse genetic approaches toward further analysis of cAMP regulated morphogenesis. The roles of two protein phosphatases (PP2A and PP2B), hypothesized to counter PKA activity, were tested by mutagenesis and/or inhibitor studies. Calcineurin (PP2B) mutants have phenotypes consistent with those predicted for a protein phosphatase with a role in dephosphorylation of PKA substrates. These included generation of cell clusters of buds defective in cell separation. Deletion mutants for a second protein phosphatase, PP2A were not identified amongst numerous transformants, suggesting that the gene may be essential. Consistent with this result was the fact that addition of the PP2A inhibitor okadaic acid at low concentration caused cells to form clusters of buds similar to hyperactivity of PKA while slightly higher concentrations killed the cells. Two other genes, *cap1*, the cyclase associated protein and *cab1*, encoding a putative cAMP binding protein have been identified and their roles in cAMP signaling are being analyzed.

161. Identification of an essential fruiting gene from a REMI mutant in the basidiomycete *Schizophyllum commune*. Stephen Horton and Ben Wormer. Department of Biological Sciences, Union College, Schenectady, NY 12308 USA

In an effort to identify new genes essential to the process of mushroom development in the basidiomycete *Schizophyllum commune*, we have utilized the REMI mutagenesis procedure. A homokaryotic fruiting strain was used as a REMI recipient: we describe here the initial characterization of one non-fruiting mutant. Flanking genomic sequence disrupted by the insertion of the transforming plasmid was rescued in *E. coli*. The mutant was determined to be the result of a true REMI event by Southern hybridization. DNA sequencing of 5' and 3' RACE clones allowed for the prediction of the corresponding polypeptide. The gene product may facilitate hyphal aggregation, based upon some limited similarity found to other polypeptides in the protein databases. The abundance of the corresponding 2 kb transcript may be regulated with regards to fruiting body development. Targeted gene disruption experiments are being performed to confirm the essential nature of this gene to the process of fruiting. We are also in the process of overexpressing this gene to test the hypothesis that this might increase hyphal aggregation, which in turn may enhance fruiting in *S. commune*.

162. Circadian Rhythms in Development and Gene Expression in Aspergillus. Andrew Greene[*1,2], Nancy Keller[3], Hubertus Haas[4], and Deborah Bell-Pedersen[1,2]. [1]Program For the Biology of Filamentous Fungi, [2]Department of Biology, Texas A&M University, College Station, TX 77843, [3]Department of Plant Pathology, University of Wisconsin, Madison, WI 53706, [4] Department of Molecular Biology, University of Innsbruck, Fritz-Pregl-Str. 3 A-6020, Innsbruck, Austria

The circadian clock controls daily cycles in biochemical, physiological, and behavioral processes and has been observed in organisms ranging from cyanobacteria to humans. Among the fungi, the circadian clock has been most extensively studied in the filamentous ascomycete*Neurospora crassa*, and a detailed understanding of the mechanisms involved in generating circadian rhythmicity has emerged. While daily and circadian rhythms have been documented in other fungi, we know relatively little about the degree of conservation of clock components and mechanisms within the fungal kingdom. In order to begin addressing these questions, we have demonstrated circadian rhythms in development and gene expression in the ascomycetes *Aspergillus flavus* and *Aspergillus nidulans*, respectively. The *A. flavus* sclerotial rhythm has an unusually long free-running period of 33 hours and entrains to light:dark cycles in a unique manner. Additionally, the *frequency* gene, required for circadian rhythmicity in *Neurospora* under normal growth conditions, is not present in the sequenced genome of *A. nidulans* nor the closely related *A. fumigatis* species. These observations suggest that the circadian clock in *Aspergillus* is organized differently from that of *Neurospora*.

163. Identification of *Neurospora crassa period-4* (*prd-4*) may link the circadian clock and cell cycle. António M. Pregueiro, Jay C. Dunlap and Jennifer J. Loros. Departments of Genetics and Biochemistry, Dartmouth Medical School, Hanover, NH 03755

Neurospora crassa is a well established model to study the circadian clock. Among other functions, the clock regulates the developmental program leading to formation of asexual macroconidiophores. This leads to a rhythm in conidiation with a periodicity of approximately 22 hours under constant conditions. In the wild-type strain, this rhythm is endogenous and self-sustained, responds to environmental signals including light and temperature, and maintains an approximately constant periodicity within the organism's physiological range of temperature. This is a fundamental property of circadian clocks and is known as temperature compensation. Several mutants defective in clock properties have been found in *Neurospora*. One of them, *period-4* (*prd-4*), has a markedly short period length of ~18.5 hours at 25C. The mutant also fails to exhibit temperature compensation, displaying an even shorter period length at higher temperatures. Heterokaryon analyses of the mutant previously showed that the *prd-4* mutation is semi-dominant to wild-type making identification of the gene by rescue extremely difficult. With the release of the *Neurospora* genome sequence, we have sequenced the *prd-4* region on Linkage Group I and found a single base-pair change that we now demonstrate is responsible for the *prd-4* mutant phenotype. The gene's sequence is consistent with a role as a mitotic cell cycle regulator and may provide an entrée to understanding gating of cell cycles and the connection between the circadian clock and cell division.

164. Isolation of a mutant with uninucleate conidia from *Aspergillus oryzae* and its use as a host strain. K. Ishi, J. Maruyama, H. Nakajima and K. Kitamoto. Department of Biotechnology, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Aspergillus oryzae forms multinucleate conidia, which are considered to contribute to genetic stability in Japanese traditional food fermentation. However, this characteristic impedes the efficiency to isolate recessive mutants and homokaryotic transformants. We[1)] previously reported visualization of nuclei by expressing histone H2B-EGFP fusion protein in *A. oryzae*. We isolated *mu*lti*n*ucleate conidia deficient mutants (*mun*$^-$) using FACS from H2B-EGFP expressing strain (*niaD*$^-$, *niaD::h2b-egfp*) by UV irradiation. An isolated mutant, uni10 (*niaD*$^-$, *niaD::h2b-egfp*, *mun*$^-$), formed the highest percentage (approximately 80%) of uninucleate conidia. It showed almost the same phenotype in growth, conidiation and secretion of extra-cellular enzymes as the parent strain, suggesting that uni10 may be a useful host strain to isolate mutants and homokaryotic transformants. After curing of *h2b-egfp* from uni10 by positive selection method, an auxotrophic strain (*niaD*$^-$, *sC*$^-$,*mun*$^-$) was bred and transformed with plasmids carrying each selection marker (*niaD* or *sC*) and heterologous genes. Therefore, it is expected that this strain can be used as a host in protein production. The efficiency of homokaryon formation after transformation will be discussed.
1) Maruyama J, *et al*. (2002). FEMS Microbiol Lett 206, 57-61

165. An ortholog of the *Saccharomyces cerevisiae* protein Prm1 is involved in mating of both heterothallic and homothallic *Cochliobolus* species. S.E. Baker*, S.-W. Lu**, O.C. Yoder*, S. Oide**, B.G. Turgeon**. *Torrey Mesa Research Inst., **Cornell Univ.

Mating of heterothallic ascomycetes involves both cell-cell recognition and fusion between cells of opposite mating type and nucleus-nucleus recognition and fusion, once cells have fused. Cell fusion is required initially when the male cell fuses with the female trichogyne and subsequently when cells in the crozier merge. Are all steps a requirement for sexual reproduction of homothallic species? The process of cell fusion is complex, requiring participation of many proteins, including, ultimately, proteins mediating the actual fusion of cell membranes. We have found orthologs of the yeast gene PRM1 encoding a putative fusase protein, Prm1p, in the filamentous heterothallic ascomycete *Cochliobolus heterostrophus* and in *C. luttrellii*, a homothallic species closely related to *C. heterostrophus*. Deletion of the gene encoding this protein in *C. heterostrophus* appears to have no effect on mating ability or on fertility; numbers of pseudothecia, asci and ascospores formed when a prm1-mutant is crossed to wild type or to a prm1-mutant of opposite mating type are as in wild type crosses. In contrast, deletion of the gene in *C.luttrellii* and selfing of transformants yields very small pseudothecia with greatly reduced numbers of mature asci (less than 10% of wild type). The asci and ascospores that are made, appear to be wild type. These results provide evidence that Prm1p may be required by homothallic fungi for cell fusion between hyphae and between crozier cells.

166. A basic-region helix-loop-helix protein-encoding gene (hpa3) involved in the development of *Aspergillus nidulans*. André Tüncher, Hans Reinke, Goran Martic, Maria Louise Caruso and Axel A. Brakhage Institut für Mikrobiologie, Universität Hannover, Schneiderberg 50, D-30167 Hannover, Germany

By colony hybridization of an Aspergillus nidulans cosmid genomic gene library using a probe which encoded the highly conserved basic-region helix-loop-helix (bHLH) domain of AnBH1, a novel gene designated hpa3 was isolated. HPA3 contains a region with high similarity to the bHLH-domain of different bHLH proteins. The highest similarity was found to an unknown ORF of A. fumigatus and some similarity to ESC1 of *Schizosaccharomyces pombe*. ESC1 is involved in the induction of sexual differentiation by nitrogen starvation. The analysis of HPA3 using the green fluorescent protein showed that HPA3 is located in the nucleus of *A. nidulans*. To study the physiological meaning of hpa3 a knock-out mutant was produced which was viable. However, it did not produce conidia on minimal agar plates. Scanning electron microscopic inspection showed that although the conidiophore including the vesicle was formed, hardly any metulae were produced. The addition of KCl (0.6 M) or sucrose (1.1 M) to the medium suppressed the knock-out phenotype. Induction of an alcAp-hpa3 gene fusion in a Dhpa3 mutant led to the wild-type phenotype of the mutant strain, whereas on repressing media (glucose) the mutant strain exhibited the Dhpa3 phenotype. Northern analysis revealed that hpa3 mRNA steady state levels were about the same in sporulating and vegetatively growing mycelia. Moreover, under all conditions tested self-crossing of the Dhpa3 mutant strain was never observed.

Gene Regulation

167. Identification and characterization of *Neurospora crassa* mutant strains that are rhythmic in continuous light. Kyung Suk Seo and Deborah Bell-Pedersen. Department of Biology, Texas A&M University, College Station, Texas 77843.

In *Neurospora crassa*, an endogenous circadian clock regulates daily rhythms of asexual spore formation. Under standard growth conditions, the developmental rhythm requires the function of the well-described FRQ oscillator (FO). The FO is composed of an autoregulatory feedback loop involving the *frq*, *wc-1* and *wc-2* genes. The FO receives input signals from the environment to synchronize the clock to the outside world, and is involved in signaling time-of-day information to the rest of the cell to control overt rhythmicity. Despite the importance of the FO in circadian timing, recent evidence has shown that under some growth conditions (e.g. in temperature cycles) circadian rhythms in development are present in strains that lack a functional FO. These data suggested the presence of an additional FRQ-less oscillator (FLO) in *N. crassa* cells. In attempts to identify components of the FLO, we have generated mutant strains that are robustly rhythmic in the absence of the FO in continuous light, and unlike strains that lack the FO, the mutant strains can be entrained in LD cycle. Genetic characterization of the mutant strains will be discussed in the context of a dual oscillator model.

168. The gene for the heat-shock protein HSP100 is induced by blue light and heat-shock in Phycomyces. Julio L. Rodriguez-Romero and Luis M. Corrochano. Departamento de Genetica, Universidad de Sevilla, Apartado 1095, E-41080 Sevilla, Spain

The Zygomycete *Phycomyces blakesleeanus* develops two types of fruiting bodies of very different size, macrophores and microphores. Blue light stimulates macrophorogenesis and inhibits microphorogenesis. To investigate the role of differential gene expression during photophorogenesis in Phycomyces we have adapted a method based on the polymerase chain reaction with arbitrary primers. With this method we have isolated a cDNA segment for the heat-shock protein HSP100 that is induced by blue-light at the onset of sporangiophore development. The gene for HSP100 (hspA) is induced by blue-light and heat-shock. The expression of hspA is induced eleven fold after 30 minutes of blue light but longer exposure times reduced its expression. A short illumination (10 seconds) induced hspA but a period of incubation in the dark was necessary to detect the mRNA. Phycomyces cultures are usually grown at 22 degrees. A heat-shock, 34 degrees during 30 min, induced the hspA gene about 100 fold but incubations at higher temperatures reduced its expression. Our results suggest that light and heat can activate the expression of hspA to different levels. Several mutants altered in the Phycomyces light responses are available. The effect of these mutations on the expression of hspA by light and heat and the isolation of its regulatory sequences will aid to establish the molecular details of photophorogenesis in Phycomyces.

169. Gene Complements Induced by Forced Shift from Glucose to Alternate Carbon Sources in *Aspergillus nidulans*.

Sunita Macwana[1] and Rolf Prade[1]. Department of Microbiology & Molecular Genetics Oklahoma State University, Stillwater OK 74078

Little is known about the extracellular enzymes *A. nidulans* produces while growing on plant cell wall polysaccharides. This study is based on a novel molecular screening method, aimed at the recovery of cDNA clones from all transcripts *A. nidulans* induces when forced to shift from glucose to a medium containing one or a range of polysaccharides, including pectin, cellulose, xylan and other plant cell wall components. cDNAs, prepared from mRNA templates extracted from glucose-grown cultures, were labeled (herein designated "glucose-grown" probes) and used to screen a cDNA plasmid library made from mRNAs extracted from plant cell wall polysaccharide-containing cultures (herein designated polymer-grown library). Isolation of condition-specific induced cDNAs was accomplished through differential DNA/DNA hybridization among a labeled "glucose-grown" probe, membrane cross-linked "polymer-grown" plasmid-clone library and negatives were selected for further analysis under the assumption that they were the ones induced as a consequence of the physiological shift. Thus, if selection of negatives is exhaustive, the suggested approach is comprehensive because a whole gene set activated by a specific physiological condition is recovered. We have isolated over 1,600 unique cDNA whose transcript does not appear to

be present in glucose growing cultures and observed that only a fraction of polysaccharide degrading enzyme coding genes were found. Moreover, the screening data have been corroborated with time-course microarray expression profiling. This unexpected outcome suggests that significant intracellular metabolic changes take place when shifting carbon sources and that the presence of extracellular polymer degrading activities is regulated differently, not involving an exclusive induction of gene expression.

170. Upregulation of promoter activity of the Taka-Amylase A gene. Kanako Suzuki[1], Yoshinao Koide[1], Masashi Kato[2], Tetsuo Kobayashi[2], Norihiro Tsukagoshi[2]. [1]Amano Enzyme Inc., Kakamigahara, Japan. [2]Nagoya University, Nagoya, Japan.

Aspergilli have been widely utilized for production of various enzymes of industrial interest because of their high productivity. We have been characterizing regulatory mechanisms underlying expression of the *A. oryzae* Taka-amylase A gene (*taa*) to construct a high level expression system by utilizing regulatory elements involved in high levels of enzyme production. The *taa* gene contains several regulatory elements such as CCAAT-box and Starch Response Element (SRE). The CCAAT-binding complex, so called Hap complex, has been proved to increase the expression levels of many genes. AmyR, SRE-binding protein, mediates the inducible expression of the amylolytic genes. We inserted a 190 bp DNA fragment carrying both CCAAT and SRE sequences to the promoter region of the *taa* gene. One of those constructs, which had the DNA fragment downstream of the SRE sequence, exhibited the highest promoter activity. Using this upregulated promoter, we expressed two fungal genes encoding Taka-amylase A (*A. oryzae*) and Laccase (*Paeaphaeoshaeria sp.*) as homologous and heterologous genes, respectively. When these genes were integrated with multiple copies into *A. oryzae*, transformants produced extracellularly a large quantity of both enzymes. This indicates that the promoter constructed here is one of the most efficient promoters for enzyme production in filamentous fungi.

171. Nuclear localization of *Aspergillus nidulans* AreA is regulated by nitrogen and carbon starvation. Richard B. Todd, James A. Fraser, Michael J. Hynes and Meryl A. Davis. Department of Genetics, University of Melbourne, Parkville 3010, AUSTRALIA

The global transcriptional regulator AreA activates transcription of many genes required for nitrogen catabolism under nitrogen limitation. AreA levels and activity are regulated autogenously, by differential mRNA turnover and interaction with the NmrA and TamA proteins. We describe an additional level of regulation of AreA activity. We have epitope tagged AreA by gene replacement at the *areA* locus and used immunofluorescence microscopy to determine the subcellular localization of AreA. Under nitrogen starvation the AreA protein hyperaccumulates in the nucleus. This correlates with a significant elevation of nitrogen catabolic gene expression. Furthermore, hyperaccumulation is NmrA-independent and does not require residues 60-423 or 854-876 of AreA. The AreA protein is not observed to accumulate in the nucleus in the presence of a nitrogen source. Transfer from nitrogen starvation to nitrogen sufficient conditions triggers rapid exit of AreA from the nucleus, consistent with the idea that AreA is transcriptionally inactive during nitrogen sufficiency. The increase in certain nitrogen catabolic enzyme levels in response to nitrogen starvation is prevented by carbon starvation. We show that simultaneous carbon starvation prevents the AreA hyperaccumulation observed under nitrogen starvation. Furthermore, transfer from nitrogen starvation conditions to carbon starvation conditions rapidly reverses AreA hyperaccumulation. These studies demonstrate that AreA activity can be differentially regulated by subcellular localization in response to distinct signals generated under nitrogen and carbon starvation.

172. A quantity control mechanism regulating levels of the HapE subunit of the Hap complex in *Aspergillus nidulans*: HapC adjusts the number of HapE. Masashi Kato and Norihiro Tsukagoshi. Department of Biological Mechanisms and Functions, Graduate school of Bioagricultural Sciences, Nagoya University, Nagoya, Japan.

The CCAAT sequence is one of the most common cis-elements present in the promoter regions in eukaryotes. *Aspergillus nidulans* CCAAT-binding complex (Hap complex) consists of at least three subunits, HapB, HapC and HapE. To investigate the quantity control mechanisms of the subunits during assembly of the Hap complex, reconstitution studies with the recombinant subunits and the extracts prepared from the respective *hap* subunit deletion mutants were carried out. Furthermore, Western blot analysis on the Hap subunits and Northern blot analysis on the *hap* genes with the respective deletion mutants were also performed. From all the results together, it was suggested that the number of the HapC molecule could adjust that of the HapE molecule by forming stable

heterodimers prior to assembly of the Hap complex. Although many CCAAT complexes have been characterized from various eukaryotes, little is known about stability of their subunits. The findings reported here suggest that similar quantity control mechanisms of the subunits may exist in the other eukaryotes. 1) M. Kato et al. FEBS Letters 512, 227-229 (2002).

173. Identification of a new gene - *suX(pro)* involved in a suppression of proline auxotrophy in *Aspergillus nidulans* using a heterologous transposon *Impala*. Anna Olszewska, Agnieszka Dzikowska and Piotr Weglenski. Department of Genetics, Warsaw University.

Transposon tagging is a very useful tool for insertional mutagenesis and gene cloning. Transposons can be employed to tag genes both in *Procaryota* and *Eucaryota*. The aim of this project is to obtain an *A. nidulans* mutant with a suppression of proline auxotrophy by using a heterologous transposon *Impala* from *Fusarium oxysporum*. *A.nidulans* initially strain was a mutant in *proA* gene, thus it had a *pro-* phenotype. Using transposon mutagenesis we obtained a suX(pro) mutant which, in spite of mutation in *proA* gene, was able to grow on a medium without proline. Inverse PCR method was used for amplification of tagged gene. The *suX(pro*) gene was amplified together with upstream and downstream regions. Further it was cloned on plasmid vector and sequenced. An open reading frame of 1335bp was identified. It contains one putative intron (92bp) into which the *Impala* element has been transposed. The putative product of the *suX(pro)* gene is a protein of 259 amino acids residues. This protein comprises two conservative RRM domains (RNA recognition motif), each of them contains two RNP-SC regions (ribonucleoprotein consensus sequence). Proteins comprising RRM domains play a crucial role in posttranscriptional regulation of gene expression at the level of poliadenylation, splicing, transport of mRNAs from nucleus to cytoplasm and in mRNAs stabilization.

174. Functional analysis of the positive-acting sulfur regulator MetR of *A. oryzae*. Genryou Umitsuki[1], Motoaki Sano[2], Osamu Hatamoto[1], Seiichi Hara[1], Tsutomu Masuda[1], Masayuki Machida[2]. [1]Noda Institute for Scientific Research, Noda, Chiba 278-0037, Japan. [2]Research Center for Glycoscience, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, 305-8566, Japan.)

A. oryzae, one of the koji mold species, has been used to produce fermented foods such as soy sauce, *miso*, and *sake*. In order to breed the improved *A. oryzae* strains and to optimize the production process of fermented foods, an understanding of various gene regulation systems of *A. oryzae* is of great importance. Analyses of the mechanisms of carbon catabolite repression and the regulation of the nitrogen assimilation genes are under progress. On the other hand, little is known about the regulation of the sulfur assimilation genes in *A. oryzae*. We have cloned *metR* gene encoding MetR protein from *A. oryzae*, which is homologous to the positive-acting sulfur regulators MetR of *A. nidulans* and CYS3 of *N. crassa*. Electrophoretic mobility shift assay revealed the specific binding of *A. oryzae* MetR to a particular sequence of a DNA fragment in vitro. An *A. oryzae* strain that expressed *metR* gene under the control of *amyB* promoter was made and characterized. This strain showed derepression of arylsulfatase, which is known as one of the sulfur-repressed gene products. This strain also showed higher extracellular protease activity than the parental strain. These results suggest that *A. oryzae* MetR is a positive-acting sulfur regulator responsible to the induction of extracellular protease expression by the limitation of sulfur source in the medium.

175. A putative *Aspergillus nidulans* transcriptional regulator from a MADS-box family. Agnieszka Dzikowska, Joanna Empel, Rafal Tomecki and Piotr Weglenski. Department of Genetics, Warsaw University, Warsaw, Poland

The arginine catabolism gene *otaA* coding for ornithine transaminase (OTAse), is specifically induced by arginine. This induction depends on two elements: the Zn2C6 transcriptional activator - product of the *arcA* gene and specific target in the promoter of *otaA* gene. In the *otaA* promoter we have identified sequences responsible for arginine induction (AnUASarg). Deletions within AnUASarg result in non-inducibility of OTAse by arginine in vivo. Moreover, the expression of *otaA* carrying deletions within AnUASarg is not activated by the ARCA^{d}47 super-activator although*arcA^{d}47* is a dominant constitutive mutation resulting in an elevated level of arginase and OTAse activity in a wild type strain. The AnUASarg sequence is very similar to *Saccharomyces cerevisiae* UASarg, a sequence present in the promoters of arginine metabolism genes that is a target for a complex of Zn2C6 activator (ArgRIIp) with two transcriptional regulators from a MADS-box family (McmIp and ArgRIp). We have identified in CEREON *A. nidulans* sequence database a MADS-box domain which is almost the same as McmIp domain. The

A. nidulans domain was amplified by PCR and the gene was cloned from *Aspergillus* Minimal Compressed Library (FGSC).

176. Functional analysis of *Aspergillus oryzae* XlnR and upregulation of its target gene expression. Junichiro Marui[1], Noriyuki Kitamoto[2], Masashi Kato[1], Tetsuo Kobayashi[1] and Norihiro Tsukagoshi[1]. [1]Nagoya University, [2]Aichi Industrial Technology Institute, Japan.

Aspergillus oryzae has been widely utilized for production of various enzymes of industrial interest because of their high productivity. *A. oryzae* XlnR was isolated as a transcriptional activator of the major xylanase gene, *xynF1*. To examine the spectrum of the xylanolytic and cellulolytic genes under the control of XlnR, their expression in an *A. oryzae* wild type strain, a *xlnR* disruptant and a *xlnR* overexpressed strain was analyzed by Northern blotting. XlnR directed expression of at least four xylanolytic and four cellulolytic genes when induced by xylan and xylose. Moreover, XlnR was newly found to mediate the cellulose-dependent expression of the xylanolytic genes as well as the cellulolytic genes in *A. oryzae*. This was further confirmed by monitoring beta-galactosidase activity in transformants carrying the authentic or mutated *xynF1* promoter controlled *lacZ* gene grown on cellulose. The *xynF1* gene contains two different affinity binding sequences for XlnR and one analogous sequence in a short promoter region. The high affinity binding sequence, 5'-GGCTAA-3', was approximately 8 times more effective in induction of the *xynF1* gene than the low affinity binding sequence, 5'-GGCTGA-3', which exhibits approximately 10 times lower affinity compared to the higher affinity sequence. Promoter activity of the *xynF1* gene was upregulated up to 2.8-fold by mutating both the lower affinity binding sequence and the analogous sequence to the higher affinity binding sequence.

177. A putative G protein-coupled receptor controls growth, germination and coordinated development in *Aspergillus nidulans*. Kap-Hoon Han, Jeong-Ah Seo and Jaehyuk Yu. Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, WI 53706 USA.

Comprehensive comparative genomic studies on heterotrimeric G-protein signal transduction components lead us to identify seven genes (*gprA~gprG*) that are predicted to encode putative seven-transmembrane spanning G protein-coupled receptors (GPCRs) in *Aspergillus nidulans*. Functional characterization of four (*gprA~gprD*) GPCRs has been carried out and disruption of *gprD* resulted in extreme phenotypic abnormalities. The *gprD* gene is predicted to encode a 427 amino acid polypeptide with typical seven transmembrane domains. The *gprD* null mutant exhibits severely reduced colony growth and highly elevated fruiting body formation, implying that the mutant fails to coordinate balanced growth and development. Moreover, the timing of spore germination of the*gprD* null mutant is delayed at least two hours, indicating GprD may also play a crucial role in germination. In order to examine whether extreme growth reduction is caused by uncontrolled activation of sexual development, double mutants of *gprD* deletion and deletion of the genes related to sexual differentiation were generated. Both double deletion mutants of *gprD*; *nsdD* and *gprD*; *veA* showed no fruiting body formation and restored hyphal growth to near wild-type level. Furthermore, environmental conditions, including poor carbon source and/or high levels of salt, that induce asexual development and block sexual development also caused the recovered growth with the absence of fruiting body in the *gprD* null mutant. These results clearly suggest that the primary role of GprD is to repress and coordinate sexual development and to confer proper growth during the lifecycle. Further studies to dissect genetic components functioning downstream of GprD are in progress.

178. FacB independent activation of *Aspergillus nidulans* isocitrate lyase (AcuD) is mediated by propionate. Matthias Brock. Department of Microbiology , University Hannover, Schneiderberg 50, 30167 Hannover, Germany

FacB is known to be the transcriptional activator of the acetate utilisation genes. Therefore, a FacB mutant of *Aspergillus nidulans* is unable to grow on acetate as sole carbon and energy source. However, isocitrate lyase activity, as a marker enzyme of the glyoxylate cycle, is induced in the presence of propionate, even in a *facB* negative background. We were able to show that a *facB* mutant is able to utilise significant amounts of acetate when grown on mixed carbon sources like acetate/propionate medium. Therefore, we postulate the existence of an alternative transcriptional activator, which is specifically induced by propionate. On the other hand, FacB seems to be able to act as a negative transriptional regulator of the propionate metabolising genes. Comparison of enzyme activities of marker enzymes of the glyoxylate and methylcitrate cycle revealed that activity of enzymes of the

methylcitrate cycle, responsible for propionate degradation, is always low in the presence of acetate. Interestingly, this regulation is much weaker in a *facB* negative mutant.

179. Core promoter structure in the oomycete *Phytophthora infestans*. Adele McLeod, Christine D. Smart, William E. Fry. Cornell University, Ithaca, NY

The core promoter structure of the oomycete *Phytophthora infestans* was investigated. The transcriptional start sites (tss) of three previously characterized *P. infestans* genes *Piexo1*, *Piexo3* and *Piendo1* were determined by primer extension analyses. Their tss regions were homologous to a previously identified 16nt core sequence that overlaps the transcriptional start site in most oomycete genes. The core promoter regions of *Piexo1* and *Piendo1* were investigated using a transient expression assay with the promoters of the genes being driven by the beta-glucuronidase reporter gene. Mutational analyses in the promoter of *Piexo1* and *Piendo1* showed that there is a core promoter element encompassing the transcriptional start site (-2 to +5) that has high sequence and functional homology to a known core promoter element present in other eukaryotes, the initiator element (Inr). Flanking the Inr is a highly conserved oomycete promoter region (+7 to +15), hereafter referred to as FPR (flanking promoter region), which is also important for promoter function. The importance of the Inr and FPR were further investigated in electrophoretic mobility shift assays (EMSA) using *P. infestans* whole-cell protein extracts, and 31-36 bp double-strand oligonucleotides containing the Inr and FPR. These studies showed that mutations in the Inr (-1 to + 3) and FPR (+9 to +11) affected protein binding negatively, and that these regions were thus important for protein binding. Furthermore, the EMSA studies showed that both the Inr and FPR were required for protein binding. Thus, both protein binding studies and transient expression analyses suggest that the FPR and Inr function cooperatively.

180. Heterokaryon incompatibility associated with the large subunit of ribonucleotide reductase in *Neurospora crassa*. Carmen Gibbs, Leila Haidari, Cristina Micali and Myron Smith. Biology Department, Carleton University, Ottawa, Ontario, Canada

The *un-24* gene of *N. crassa* encodes the large subunit of a type I ribonucleotide reductase (RNR), an evolutionarily conserved enzyme that is essential for *de novo* DNA synthesis. UN-24 is remarkable among type I RNRs in having heterokaryon incompatibility function in addition to RNR catalytic activity. To understand how *un-24* mediates heterokaryon incompatibility, we are examining incompatibility and catalytic activity of truncated and chimeric *un-24* constructs, as well as *un-24*-derived transcript and protein levels from strains that are self-incompatible due to heteroallelic duplications of *un-24*. Our results indicate that incompatibility activity is associated with a C-terminal domain unique to *N. crassa* that also differs between the two allelic forms (*un-24*^{PA} and *un-24*^{OR}), but that incompatibility is not dependent on RNR catalytic activity. Surprisingly, transcription of *un-24*^{PA}, but not *un-24*^{OR}, is upregulated in haploid strains grown on a low nitrogen medium that promotes the sexual cycle; therefore, localized inactivation of *un-24*-mediated incompatibility in the perithecium may be through differential transcript regulation of the two allelic forms. Preliminary Western analyses indicate that UN-24 proteins accumulate and form dimer aggregates in self-incompatible *un-24*$^{OR/PA}$ partial diploids suggesting that *un-24*-associated incompatibility may be a non-specific response to the presence of protein aggregates rather than a modification of RNR activity.

181. Genetic Regulation of Cellular Copper Homeostasis in *Podospora anserina*: Impact on Oxidative Stress. Heinz D. Osiewacz. J.W. Goethe University, Botanical Institute, Frankfurt/ Main, Germany

In biological systems, copper is an essential trace metal that is required as a cofactor of different enzymes (e.g., cytochrome oxidase, Cu/Zn superoxide dismutase). However, elevated cellular copper levels are toxic since this metal, like iron, leads to the generation of the highly toxic hydroxyl radical. Moreover, electron transport following the copper-dependent cytochrome oxidase pathway at the inner mitochondrial membrane are known to lead to a time-dependent increase of mitochondrial oxidative stress and to degenerative processes. The dual role of copper makes it essential to tightly control cellular copper levels. In*Podospora anserina* this is achieved via the copper-regulated transcriptional control of different genes. Among others, these are genes encoding a copper metallothionein (PaMT1) and a high affinity copper transporter (PaCTR3). The expression of the latter is controlled by GRISEA, a transcription factor that becomes inactivated at increased cellular copper levels. The presented data and those from earlier investigations will be discussed and compared to those from other systems demonstrating that basic parts of the machinery involved in the control of copper homeostasis appear to be conserved among organisms. However, others parts differ allowing a flexible adaptation of the corresponding organism to specific

ecological niches. The experimental work is supported by grants of the Deutsche Forschungsgemeinschaft (Bonn, Germany)

182. Insertional Mutagenesis Using *Agrobacterium tumefaciens* T-DNA to Identify Transcriptional Regulators of the *Blastomyces dermatitidis BAD1* Promoter. Julie C. Nemecek[1], Thomas D. Sullivan[2] and Bruce S. Klein[1, 2, 3, 4]. Departments of [1]Medical Microbiology and Immunology, [2]Pediatrics, and [3]Internal Medicine and the[4]Comprehensive Cancer Center, University of Wisconsin, Madison, Wisconsin.

The *BAD1* gene is a yeast-phase specific virulence factor of the dimorphic, animal pathogen *B. dermatitidis.* With the eventual goal of delineating the factors necessary for regulating phase transition, we have begun a genetic search for regulators of the yeast-phase expression of *BAD1.* A reporter strain was generated expressing the *E. coli LacZ*gene from the *BAD1* promoter. In this strain beta-galactosidase activity is produced at high levels in yeast cells, resulting in blue colonies in the presence of Xgal. The reporter strain was subjected to transformation using *A. tumefaciens* T-DNA as an insertional mutagen. Thus far 11,841 primary transformants have been screened by staining with Xgal, and 478 white colonies were picked. Of these, 312 were reproducibly white or light blue in color after long exposure to Xgal. For 39 of 284 tested there was low or undetectable secretion of authentic BAD1 protein, and for 4 of 14 tested there were low levels of extractable beta-galactosidase activity. Promising lines will be further analyzed by Southern blot for the number of sites of T-DNA insertion in the genome. Those with single sites will be selected to use for amplification and determination of the sequence flanking the T-DNA and to confirm that the steady state level of BAD1 transcript is decreased in the presence of the mutation.

183. Functional analysis of the Aspergillus nidulans xprF gene, a Gene Involved in the Response to Starvation. Bernardo, S.M.H., Cheetham, B.F., Katz, M.E. *Molecular and Cellular Biology, University of New England, Armidale, NSW, Australia*

The *Aspergillus nidulans xprF* gene appears to be involved in carbon starvation. The gene is unique in that it is involved in both carbon regulation of extracellular protease production and the utilisation of certain nitrogen sources. Sequence analysis shows that the predicted gene product of 615 amino acids residues is a hexokinase-like protein, sharing highly conserved residues within the ATP- and sugar-binding domains of other well-established hexokinases. A number of hexokinases in plants, mammals, and fungi have been shown to play a regulatory role in glucose repression, but no role in carbon starvation has been reported. The *xprF1* mutation is a nonsense mutation predicted to result in a truncated protein of 255 amino acid residues. The *xprF-delta* mutant created in this study exhibited a similar phenotype to the *xprF1* mutant in terms of regulation of extracellular proteases and utilisation of nitrogen sources. Similarly, the knockout mutant exhibited partial dominance; this shows that the partial dominance exhibited by the *xprF1* mutation is not due to a dominant-negative effect of the truncated protein. Using site-directed mutagenesis, we have shown that a number of unusual, as well as conserved, features of the *xprF* gene product are important for the maintenance of its functional integrity. Deletion of the unique sequences at the N- and C- termini showed that both domains were required for XprF function. It has also been shown that a number of highly conserved residues in the ATP-binding domains are not required for its regulatory function. Mutations in a putative nuclear localisation domain also affected gene function. The *xprF* gene has been expressed in E. coli in order to purify the protein and subsequently test for hexokinase activity. Nuclear localisation experiments, using *xprF*-gfp fusions, are also underway.

184. The role of carbon catabolite repression, carbon starvation and induction by exogenous protein in the regulation of extracellular protease production in *Aspergillus nidulans*. Margaret E. Katz and Brian F. Cheetham. Molecular and Cellular Biology, University of New England, Armidale, NSW, Australia.

Extracellular protease production was analysed in *A. nidulans* strains carrying mutations in genes thought to be involved in the response to carbon starvation (*xprF and xprG*) and genes which play a role in carbon catabolite repression (*creA, creB and creC*). In wild type strains, extracellular protease levels are low when any carbon source, including protein, is present suggesting that the extracellular protease genes are only expressed at high levels when no carbon source is available. No evidence for induction by exogenous protein in the presence of repressing or non-repressing carbon sources was observed in a wild type *A. nidulans* strain but protein does appear to affect production in some mutants. Though there was little difference in the levels of extracellular proteases in repressing and non-repressing carbon sources, mutations in *cre* genes affect production of extracellular proteases, suggesting that these

genes may also be involved in the response to carbon starvation. Repression of extracellular proteases by glucose was not affected by deletion of the *creA* gene. The possibility that the *xprF* and *xprG* gene products play a direct role in the response of extracellular protease genes to carbon starvation is being explored by studying subcellular localisation of the *xprF* gene product in wild type and *xprG* mutant strains.

185. Two DNA-binding transcription factors involved in fruiting-body formation in the basidiomycete *Lentinula edodes* and their target genes. Kazuo Shishido[1], Yasumasa Miyazaki[2], Toru Jojima[3], Takeshi Ono[1], Yuta Sakuragi [1], and Takashi Yamazaki[1].[1]Tokyo Institute of Technology, Life Science, Yokohama, Japan.[2]Forestry and Forest Product Research Institute, Appl. Microbiol., Tsukuba, Japan.[3]Kasetsart University, Bangkok, Thailand.

The two DNA-binding transcription factors, PRIB and Le.CDC5 are involved in fruiting-body formation of *L. edodes* (Shiitake). PRIB (565 amino acids) contains a Zn(II)2Cys6 zinc-cluster motif followed by bZIP-like motif. The consensus sequence of PRIB binding was determined to be 16 bp 5'GGGGGGGACAGGANCC3'. The upstream region of*priB* gene contained four 16 bp consensus-like sequences to which PRIB actually binds, suggesting the autoregulation of *priB*. The *uck1* (UMP-CMP kinase) gene present downstream of *priB* was also suggested to be regulated by PRIB. The *uck1* gene was found to be most actively transcribed in hymenophore, especially both in the hymenium and in the divergence points of trama cells into subhymenium, of mature fruiting bodies. Genomic binding-site cloning experiment revealed the presence of another PRIB target gene, a homologue (named *mfbC*) of *S. cerevisiae YJL070C* gene whose function is unknown. The *mfbC* and *YJL070C* genes exhibited a partial homology to that of *S. cerevisiae* SEN3 protein, a regulatory subunit of the proteasome. The *mfbC* gene was shown to be actively transcribed in mature fruiting bodies, implying that *mfbC* may play a role at the final stage of fruiting-body formation. Le.CDC5 (842 amino acids) is the product of *Le.cdc5* gene which is a homologue of *Sch. pombe cdc5* gene and present adjacent to *priB*. It contains a Myb-type DNA-binding domain, a putative proline-rich activation domain and a possible leucine zipper. The consensus sequence of Le.CDC5 binding was determined to be 5'GCAATGT3'. The genomic binding-site cloning experiment reseulted in isolation of 3.7 and 3.9 kb *Hin*dIII fragments. These two fragments were shown to contain two or more 7 bp consensus-like sequences. For the two fragments, analysis of the gene present just downstream of the 7-bp sequences is in progress.

186. Intron-dependent mRNA accumulation of the *Coriolus hirsutus* lignin peroxidase gene in *C. hirsutus*. Takashi Yamazaki[1], Yutaka Okajima[1], Akira Tsukamoto[2], Jun Sugiura[2] and, Kazuo Shishido[1].[1]Tokyo Institute of Technology, Life Science, Yokohama, Japan.[2]Oji Paper Co. Ltd., Adv. Technol. Research Lab., Shinonome, Tokyo, Japan.

We have constructed the chromosome-integrating vector (MIp30) carrying the *C. hirsutus* glyceraldehyde-3-phosphate dehydrogenase gene (*gpd*) promoter -*Lentinula edodes priA* gene terminator and the selectable marker of *C. hirsutus ARG1* gene. The *C. hirsutus* lignin peroxidase (LiP) cDNA (*lip*c) was fused between the promoter and terminator of MIp30. The resulting plasmid (MIp30-lipc) was introduced into protoplasts of monokaryotic *C. hirsutus arg1* strain, followed by selection of Arg^+ Lip^+ colonies. Southern-bolt analysis revealed that all of the Arg^+ Lip^+ transformants possess several copies of the promoter-*lip*c-terminator expression cassette on their chromosomes. Northern-blot analysis, however, showed that these transformants do not contain so many mRNA molecules as detected by Northern-blot analysis. On the other hand, all of the Arg^+Lip^+ transformants obtained by introduction of the *lip* gene (contains 6 small introns) in place of the *lip* cDNA contained larger amounts of mRNA detectable by Northern-blot analysis. The results strongly suggest that the introns are required for the accumulation of mRNA. To investigate how many introns are necessary for it, two different*lip* constructs were prepared: construct-1 consists of the first exon - first intron - the appropriate cDNA sequence; construct-2, the first-third exons and introns - the appropriate cDNA sequence. So far we have analyzed the mRNA accumulation for construct-2, resulted in showing that efficient mRNA accumulation occurs by at least three introns. Analysis for construct-1 is in progress.

187. The genetic regulation of fatty acid metabolism in *Aspergillus nidulans*. Khew GS, Murray SL, Davis MA, Hynes MJ.Department of Genetics, University of Melbourne, Victoria 3010, Australia.

FacB, in *Aspergillus nidulans*, is a Zn(II)2Cys6 protein required for acetate induction of enzymes necessary for acetate utilisation. Many of these enzymes are also necessary for the utilisation of short and long chain fatty acids. However, we have found that fatty acid induction of these enzymes is independent of FacB-mediated induction. Nothing is known about the regulatory genes involved in fatty acid induction in filamentous fungi. The *acuJ* gene

encodes a carnitine acetyltransferase essential for shuttling acetyl-CoA between the mitochondria and peroxisomes and is thus required for growth on both acetate and fatty acids. The *acuJ* gene is acetate and fatty acid induced. A 300bp region of the *acuJ* 5' UTR is sufficient for fatty acid induction but not acetate induction of a *lacZ* reporter construct. This region contains a sequence with similarity to the oleate response element (ORE) necessary for fatty acid induction via the Oaf1p and Pip2p activators of *Saccharomyces cerevisiae*. We are determining whether the element is necessary for fatty acid induction. We have cloned a potential orthologue of the *OAF1* and *PIP2* genes. Futhermore, we have isolated a number of butyrate non-utilising mutants. Two of the corresponding genes have been cloned. One is an orthologue of the *PEX6* gene of *S. cerevisiae* which is involved in peroxisome biogenesis. The other encodes a Zn(II)2Cys6 activator involved in induction by short chain but not long chain fatty acids.

188. New aspects concerning nitrogen regulation of gibberellin biosynthesis in *Gibberella fujikuroi*. Bettina Tudzynski, Martina Mihlan, and Sabine Teichert. Westfälische-Wilhelms-Universität, Schlossgarten 3, 48149 Münster, Germany

The gibberellins (GAs) are a group of phytohormones, which are produced by the rice pathogen *G.fujikuroi*. After cloning the seven genes involved in GA biosynthesis, we are now interested in detailed analysis of regulation of gene expression. Six of the seven genes are repressed by high amounts of nitrogen, whereas the expression of the *P450-3* gene, encoding the last pathway enzyme, is not affected by nitrogen. The expression of GA genes is drastically reduced in *areA* mutants. In vivo *gus* reporter gene analysis with the*P450-4* promoter showed, that only some of the 10 GATA-motifs are responsible for the AREA-mediated up-regulation. Surprisingly, the replacement of the *G. fujikuroi nmr* gene (*nmr-GF*), which is homologous to the *N.crassa nmr1* gene encoding the negative acting AREA-binding regulator, NMR1, did neither result in derepression of GA biosynthesis nor in significant derepression of the nitrate reductase. Overexpression of *nmr-GF* under control of the strong promoter of the *G. fujikuroi* glutamine synthase gene resulted in slight repression of nitrate reductase but not of GA biosynthesis. Interestingly, the *G. fujikuroi* NMR is able to complement *nmr1/nmrA* mutants of *N.crassa* and *A.nidulans*, respectively, despite the neglectible function in *G. fujikuroi*. Furthermore, we want to identify the components of nitrogen sensing and signalling pathways involved in nitrogen metabolite repression. Thus we study the role of ammonium permeases and the *G. fujikuroi* TOR protein on one hand and glutamine synthase and other enzymes involved in biosynthesis of glutamine and glutamate on the other hand, in nitrogen-mediated repression of GA biosynthsis pathway.

189. Chromatin remodelling of the *alcA* promoter region of the ethanol utilization pathway in *Aspergillus nidulans* requires both a specific activator AlcR and a global repressor CreA. Igor Nikolaev[1], Martine Mathieu, and Béatrice Felenbok. Institut de Genetique et Microbiologie, Universite Paris-Sud, UMR 8621 CNRS, Centre d'Orsay, Orsay 91405, France[1] Present address: Danisco Innovation, Langebrogade 1, DK 1001 Copenhagen, Denmark

The ethanol utilization pathway in *A. nidulans* is one of the strongest inducible expression system among filamentous fungi. The *alcR-alcA* system is widely used at both fundamental and applied levels. It is strictly transcriptionally regulated both by specific induction mediated by the zinc binuclear cluster activator AlcR and carbon catabolite repression operating via the general repressor CreA. Molecular mechanisms whereby induction and repression are set up and interact have been studied in great detail. In order to determine if the level of transcription is correlated to the structural organization of the chromatin, we investigated nucleosomal rearrangements in the alcA promoter under different growth conditions. We utilized a range of *alcR* and *creA*mutants isolated either by classical or reverse genetic methods. In the absence of the inducer and under repressed growth conditions, nucleosomes are positioned within the *alcA* promoter. Under induced conditions, total depositionning occurs. In fact, AlcR binding to its DNA cognate targets upon induction is essential for removal of one nucleosome placed upstream of the closely linked direct and inverted repeat sites. To remove others, the intact activation domain of AlcR is required. For reseting the original nucleosome pattern observed upon repression and maintenance of a "chromatin-closed" state of the *alcA* promoter under non-induced conditions, both the DNA binding domain of CreA and its C-terminal region with a local homology to the effector domain of Mig1 from *Saccharomyces cerevisiae*, are necessary. Interestingly, we found that AlcR predominates over CreA for nucleosomal rearrangements. The interplay between the activator AlcR and the repressor CreA occuring at the transcriptional level corresponds to global chromatin remodeling in which both regulators are involved.

190. The nicotinic acid utilisation cluster of *Aspergillus nidulans*. Fernandez-Martin R., Cultrone A and Scazzocchio C. IGM Universite Paris-sud Bat. 409. 91405 Orsay France

The *hxnS* gene of *Aspergillus nidulans* codes for a rather extraordinary enzyme called Purine hydroxylase II (PHII). More than 20 years ago it was shown that it is a Molybdoflavoprotein, related to xanthine dehydrogenase, able to accept hypoxanthine but not xanthine as substrate. This enzyme is induced by nicotinate (actually by a product of its oxidation) and it has a low but physiologically significant nicotinate hydroxylase activity. The *hxnS* gene and the cluster where it maps, has been cloned. Surprisingly, all the amino acids thought to be involved in substrate recognition in xanthine dehydrogenase are conserved in PHII. At least 5 ORFs are induced by nicotinate. Regulatory mutations, both constitutive and non-inducible, map in a single gene at the cluster, *hxnR*, encoding a 2Cys2His Zn finger transcription factor. One ORF, *hxnP*, is highly similar to the characterised nicotinate transporter of *S. cerevisiae*. Other two ORFs, with similarities with characterised oxydo-reduction enzymes, are probably involved in furthers steps at nicotinate utilization as nitrogen source. All these ORFs are under the control of *hxnR*. This work has been funding by XONet European Project HPRN-CT-1999-00084

191. Analysis of *yps-3* expression in *Histoplasma capsulatum*. Parul Trivedi, [1] Thanh T. Hoang, [1] George S. Deepe, Jr.,[2] and

Jon P. Woods [1]. University of Wisconsin Medical School, [1] University of Cincinnati College of Medicine [2]

The *yps-3* gene is expressed exclusively by the pathogenic yeast morphotype of the dimorphic fungus *Histoplasma capsulatum*, and encodes a protein found in the cell wall and culture supernatant during in vitro growth. We used transcript (Northern blotting, kinetic PCR) and protein (Western immunoblotting, flow cytometry) detection techniques to evaluate expression during infection. *yps-3* transcript was upregulated 3-4 fold during intracellular infection of activated RAW264.7 macrophages, and 10-13 fold in mouse lung and 47-50 fold in mouse spleen after respiratory infection. Protein upregulation was also detected during activated macrophage infection. We used flow cytometry to demonstrate surface exposure of this protein, which differed considerably among different strains. This interstrain variation was confirmed by Western immunoblotting. Although the function of *yps-3* is not known, its pathogenic yeast phase-specific expression and variable expression in strains differing in virulence have been proposed as consistent with a role in pathogenesis. Our results further support an adaptive role for *yps-3* in the infection environment and furthermore suggest additional regulation beyond that associated with morphogenesis.

192. Overlapping Sense and Antisense Transcripts at the Ran1 Locus in *Histoplasma capsulatum*. Julia Z. Ng, Diane M. Retallack, and Jon P. Woods. University of Wisconsin-Madison

Histoplasma capsulatum (Hc) is a thermally dimorphic fungal pathogen that is the causative agent of the respiratory and systemic disease histoplasmosis. This organism resides and replicates in mammalian host phagolysosomes. In vivo expression technology (IVET) was performed to trap promoters that were upregulated during infection of mice and RAW 264.7 macrophages. One IVET-isolated promoter upregulated expression of a small non-polyadenylated transcript during infection of RAW264.7 macrophages. On the overlapping reverse-orientation strand, a 2.6kb transcript was expressed at equal levels during growth in vitro and infection of RAW264.7 macrophages. This polyadenylated transcript contains an open reading frame that encodes a putative protein with sequence similarity to *Schizosaccharomyces pombe* Ran1. *S. pombe* Ran1 is a serine-threonine kinase which is a negative regulator of meiosis. Episomal expression of *H. capsulatum* Ran1 functionally complemented a *S. pombe* temperature-sensitive *ran1* null mutant. We are currently testing *H. capsulatum* Ran1 mutated genes for function in the *S.pombe* complementation assay. In addition, we are examining Hc Ran1 expression under various environmental conditions, and a possible role for Ran1 in virulence.

193. Differential regulation of the *Histoplasma capsulatum* chitin synthase G gene. Clayton H. Johnson, Nori Watson, and Joan E. McEwen. J.L. McClellan VA Hospital and University of Arkansas for Medical Science, Dept. of Geriatrics and Dept. of Microbiology and Immunology, 4300 W. 7th St. (VAMC151/LR), Little Rock, AR 72207

Histoplasma capsulatum, a mammalian fungal pathogen, is exposed to oxidants produced by neutrophils and activated macrophages of the host. While screening an H. capsulatum cDNA library for oxidative stress inducible

genes, we isolated a truncated clone coding for H. capsulatum chitin synthase G (HcchsG) enzyme. Dose-dependent oxidative stress inducibility of the *HcchsG* gene was confirmed by northern blot analysis of RNA isolated from yeast cells exposed to hydrogen peroxide. Additional studies of *HcchsG* gene expression indicated that abundance of HcchsG mRNA is also regulated by carbon source (glycerol vs. glucose) and yeast vs. mycelial growth conditions. HcchsG mRNA abundance was highest in yeast grown on glycerol and lowest in mycelia, regardless of carbon source. In addition to the truncated cDNA clone, we isolated a genomic clone containing the entire*HcchsG* gene. The *HcchsG* gene codes for a protein with a predicted mass of 101,300 Daltons and isoelectric point of 8.85. A novel finding about the *HcchsG* gene is the presence of an intron that is significantly larger and/or missing from other fungal *chsG* genes. Reinforcement of the fungal cell wall, by increasing the content of chitin, may confer resistance to host-mediated defenses. Ongoing experiments, in an attempt to test this hypothesis, include the construction of recombinant expression systems and plasmid constructs for creating HcchsG over-producing and knock-out strains, respectively.

194. The two Neurospora Dicer proteins are required for "quelling" and development. Catalanotto C, Pallotta M, Braccini L and Cogoni C. Dept of Biology, Universita' La Sapienza, Rome, Italy.

In animals, the double stranded RNA-specific endonuclease Dicer produces two classes of functionally distinct, tiny RNAs: microRNAs (miRNAs) and small interfering RNAs (siRNAs). miRNAs regulate the expression of endogenous protein-coding genes at the level of mRNA translation, whereas siRNAs direct RNA destruction via the RNA interference (RNAi) pathway. In the fungus *Neurospora crassa*, trangenes trigger a post-transcriptional gene silencing phenomenon called quelling that has been shown to be mechanistically similar to RNAi. Two genes encoding Dicer–like proteins are present in *Neurospora crassa* genome. In this work we show that a double stranded RNA (dsRNA) RNaseIII-like processing activity is present in Neurospora extracts. This activity is ATP-dependent and does not require the presence of transgenes or functional qde (quelling defective) genes. Moreover, we show that a dicer1 and dicer2 double knock-out mutant is defective in transgene-induced gene silencing, suggesting that in this fungus the function of Dicer in quelling is redundant. The involvement of Dicer proteins in quelling also supports the notion that common genetic mechanisms exist between RNAi and transgene-induced gene silencing. Additional observations suggest that both DICER1 and DICER2 are required for the formation of female sexual structures. This result suggests that the Dicer activity is essential for miRNA maturation in fungi like in animals and plants.

195. Comparative sequencing of the *qa-1S* gene in *Neurospora africana* and *Neurospora crassa*. Diana R. Arnett[1] and David K. Asch[1,2]. [1]School of Biomedical Sciences, Kent State University, Kent, Ohio and [2]Department of Biological Sciences, Youngstown State University, Youngstown, Ohio.

While the quinic acid (*qa*) gene cluster of *Neurospora crassa* has been studied fairly extensively, very little is known about the *qa* gene cluster of *Neurospora africana*. One study attempted to determine the degree of conservation between several different species of *Neurospora*, culminating in sequencing *qa-x-qa-2* intergenic region of *N. africana* (Asch, et al 1991). It was also discovered that *N. africana* and *N. crassa* demonstrate significantly different restriction fragment length polymorphisms (RFLPs) in this region. Because of the usefulness of the *qa* cluster in studying transcription regulation and carbon catabolite repression, sequencing the *qa* genes and regulatory regions in other species of *Neurospora* is important in studying their regulation. We have sequenced the *qa-1S* gene of *N. africana* and compared it to that of *N. crassa* and to the *qutR* gene of*Aspergillus nidulans* (teleomorph, *Emericella nidulans*).
Asch, D.K., Orejas, M., Geever, R.F., and Case, M.E. (1991) Comparative studies of the quinic acid cluster in several *Neurospora* species with special emphasis on the *qa-x-qa-2* intergenic region. *Mol. Gen. Genet*. **230**: 337-344.

196. The *Aspergillus nidulans* ambient pH signal transduction pathway protein PalI is a plasma membrane protein that is transported to vacuoles in response to alkaline pH. Kimberly Schoenly, Matthew Warrick and Steven H. Denison. Eckerd College, Natural Sciences, St. Petersburg, FL, USA.

Regulation of gene expression by ambient (extracellular) pH in *Aspergillus nidulans*is mediated by a signalling pathway composed of the products of the *palA, B, C, F, H* and*I* genes and the PacC zinc-finger transcription factor. This pH regulatory system ensures that extracellular enzymes (as well as permeases and exported metabolites) are

produced under conditions of pH where they can function: acid phosphatase under acid conditions and alkaline phosphatase under alkaline conditions, for example. It has previously been shown that the predicted *palI* protein contains four putative membrane-spanning domains. This suggests the possibility that PalI is located in the plasma membrane and may be the first protein in the pH signalling pathway, functioning as a pH-sensing protein. We have constructed a strain of*Aspergillus nidulans* that expresses a PalI-Green Fluorescent Protein (GFP) fusion protein under control of the ethanol-inducible *alcA* promoter. Using this strain we show that PalI has a plasma membrane location, strengthening the possibility that PalI is the pH-sensor protein in the pH signal transduction pathway. When cells are transferred from acidic to alkaline medium, the PalI-GFP fusion protein appears in vacuoles within five minutes. Endocytosis of PalI may therefore be involved in pH signalling.

197. Effects of metals on the expression of the laccase gene (*Cs-lcs1*) in the ligninolytic fungus *Ceriporiopsis subvermispora*. Luis F. Larrondo[1*], Rubén Polanco[1#], Marcela Ávila[1], Augusto Manubens[1&], Paulo Canessa[1], Loreto Salas[1] and Rafael Vicuña[1]. [1]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile and Millenium Institute for Fundamental and Applied Biology (MIFAB).

C. subvermispora is a white rot fungus which is highly selective in the degradation of lignin when growing on wood. Its ligninolytic system is composed by manganese peroxidase and laccase. These activities are induced by Mn^{2+} and Cu^{2+}, respectively. Other metals such as Cd^{2+}, Zn^{2+} and Ag^{+} do not increase either of these enzymatic activities. However, Northern blot analysis showed that transcript levels of the gene coding for laccase (*Cs-lcs1*) are specifically increased in the presence of Cu^{2+}, Ag^{+} and Cd^{2+}, but not by addition of Zn^{2+}. The promoter region of *Cs-lcs1* contains putative ACE and MRE-like sequences, which have been described in other eukaryotes as recognition sites for transcription factors activated by metals. Electromobility shift assays (EMSA) revealed that nuclear proteins from this fungus bind to portions of the *Cs-lcs1* promoter which contain the aforementioned sequences. This DNA-protein interaction is specific and it seems to depend on the day of culture of the organism and on the concentration of Mn^{2+} in the culture medium. This interaction is improved when a pulse of Cu^{2+} is given 24 h. before harvesting. In addition, using the *Phanerochaete chrysosporium* genome database, we have identified a putative ACE1 like gene in this organism and the corresponding homologue in*C. subvermispora*. The transcriptional effect induced by Cu^{2+} and Ag^{+} over *Cs-lcs1*, the presence of a putative ACE sequence in its promoter and the characterization of a putative ACE-1 like gene in *C. subvermispora* suggest the existence of a regulatory mechanism similar to the one described in yeast and it would constitute the first one reported in a basidiomycete. Financed by grants 8990004, 2000076 and 2000088 from FONDECYT and the MIFAB[*]Predoctoral Fellow from Fundacion Andes. [#]PreDoctoral Fellow from MIFAB. [&]Predoctoral Fellow from CONICYT.

198. Further characterization of the *PMK1* MAP kinase pathway in *Magnaporthe grisea*. C. Xue, X. Zhao, L. Li, Y. Kim, G. Park, J. Xu. Purdue Univ. West Lafayette, IN 47907

The *PMK1* MAP kinase, a homolog of yeast Fus3/Kss1, is known to be essential for appressorium formation and plant infection in *M. grisea*. In this study we functionally characterized several candidate components of the Pmk1 pathway. Because *mst7* and *mst11* mutants failed to form any appressorium, Mst7 and Mst11 are likely to be the upstream MAPKK and MAPKKK activating Pmk1. Phenotypically, *mst7* mutants were similar to that of *pmk1* mutants, but *mst11* had additional defects in conidiation and hyphae growth, indicating that *MST11* may have additional downstream targets. Surprisingly, gene replacements of PAK kinase genes *MST20* and *CHM1* were not defective in appressorium formation. The *mst20* mutants had no obvious defect in vegetative growth and plant infection. It is likely that the Pmk1 MAPK cascade is not activated by PAK kinases in *M. grisea*. We also identified two putative homologs of yeast pheromone receptors Ste2 and Ste3 in the *M. grisea* genome (*MST2* and*MST3*). Primary data indicated that *mst2* and *mst3* mutants were normal in appressorium formation and plant infection and female fertile. In addition, we are in the process of screening for knockout mutants of yeast Ste5 and Ste50 homologs. Overall, our data indicate that the signal input and output of the *PMK1* pathway in *M. grisea* is different from that of the yeast Fus3/Kss1 pathways. Identifying other components of the Pmk1 pathway is under the way.

199. Meiotic Silencing by Unpaired DNA (MSUD). Patrick K.T. Shiu[1], Namboori B. Raju[2], Denise Zickler[3], and Robert L. Metzenberg[1]. [1]Chem and Biochem, UCLA, CA. [2]Biol Sci, Stanford University, CA. [3]Institut de Genetique et Microbiologie, Universite Paris-Sud, Orsay, France.

A gene unpaired with a homolog in prophase I generates a signal that transiently silences all sequences homologous to it by a process called Meiotic Silencing by Unpaired DNA (MSUD; Cell 107:905-916). A mutant called *Sad-1* (Suppressor of ascus dominance) fails to perform MSUD. *Sad-1* also suppresses several classical ascus-dominant mutants, suggesting that these, too, owe their ascus dominance to the MSUD mechanism. MSUD is not restricted to a few ascus-dominant genes, but is applicable to virtually the entire genome. This can be shown by the fact that a variety of genes can be meiotically silenced if they are unpaired during meiotic prophase. The *sad-1* gene encodes an RNA-directed RNA polymerase (RdRP). RdRP has been implicated in many post-transcriptional gene silencing systems, such as co-suppression in plants, RNA interference in animals, and quelling in fungi. Owing to its ability to compare the genomes of two mating partners, MSUD has implications not only for surveillance against invading sequences but also for reproductive behavior. For example, interspecific crosses within the genus Neurospora that are normally almost completely infertile become much more fertile if the *N. crassa* parent carries the *Sad-1* dominant mutation. This suggests that MSUD triggered by numerous small mispairings could play a role in reproductive isolation of these species.

200. Protein secretion stress in *Aspergillus niger*. Hashem Al-Sheikh, David Jeenes[1], Adrian Watson, Marcos Alcocer, Peter Punt[2] and David Archer. School of Life and Environmental Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK. [1]Institute of Food Research, Norwich Research Park, Norwich NR4 7UA. [2]TNO Nutrition and Food Research, PO Box 360, 3700 AJ Zeist, The Netherlands.

Aspergillus niger secretes many native proteins at high yields but the yields of most heterologous proteins are comparatively low. We have investigated the bottlenecks in protein secretion and one major bottleneck is the folding of proteins within the lumen of the endoplasmic reticulum (ER). Cells sense the presence of unfolded proteins in the lumen of the ER and the cells then respond in ways that are generally conserved within the eukaryotes. The unfolded protein response (UPR) is well described and leads to the transcriptional up-regulation of genes encoding ER-resident foldases and chaperones. The UPR also stimulates ER-associated protein degradation (ERAD) and, in mammalian cells but not shown in fungi, attenuates translation. We have reported a further response to unfolded proteins in the ER that leads to the selective transcriptional down-regulation of genes encoding secreted proteins (but not genes encoding non-secreted proteins). We report here our studies to elucidate the mechanism of this ER stress response which does not appear to be part of the UPR. This work was funded by the EC Eurofung Programme, by the Saudi Government and by the BBSRC

201. New insights into pH regulation of aflatoxin production by *Aspergillus* species. Kenneth C. Ehrlich and Peter J. Cotty, Southern Regional Research Service, ARS, USDA, New Orleans LA 70179-0687

Aflatoxins (AF) are toxic and carcinogenic metabolites of *Aspergillus* species. Previous studies found AF production to be pH dependent and, in some cases, pH inhibition and stimulation of AF production was found to override nutritional influences on aflatoxin accumulation. Differential regulation of AF production in ammonia-based media was previously found between West African (S_{BG}) and North American (S_B) strains. We now show that this differential effect results from inhibition of toxin production by the former isolates at pH 2.5, conditions in which S_B isolates produced high levels of toxin. Like *A. parasiticus* , S_{BG} isolates produce both B and G aflatoxins. Aflatoxin G production varied more with pH than did aflatoxin B production. At pH 3.5 the AFB1/AFG1 ratio was 3.0, whereas at pH 5.5 the ratio was 0.5. Although *A. parasiticus* isolates produced high levels of AF at both pH 2.5 and 5.5, the AFB1/AFG1 ratio was similarly influenced by pH. Quantitative PCR was used to examine the influence of pH on expression of six genes involved in AF biosynthesis, including two genes (*fasA* and *pksA*) for the initial steps in biosynthesis, the transcriptional regulator gene (*aflR*), and three genes for the final biosynthetic steps (*omt1, ordA*, and *ordB*). For the S_{BG} isolate, expression of most examined genes was inversely proportional to the pH affect on AF accumulation. However, reductions of *omt1* expression were paralleled by reductions in aflatoxin production at low pH. However, for the other isolates, pH effects on gene expression did not correlate with influences on AF accumulation. These results suggest that expression of *omt1* may explain a portion of the influence of low pH on aflatoxin production by S_{BG} isolates, but that most influences of pH do not occur at the level of transcription.

202. Isolation and characterization of a new gene, *pco-1*, which encodes a regulatory protein that controls purine degradation in *Neurospora crassa*. Ta-Wei D. Liu, and George A. Marzluf, Ohio State University, Columbus, Ohio

A feature of the nitrogen regulatory circuit in filamentous fungi is that pathway-specific control genes mediate induction of enzymes by substrates in specific pathways. The gene encoding a new pathway-specific factor involving in purine degradation pathway - *pco-1* was isolated from *Neurospora* using a PCR-mediated method. The open reading frame of the new factor gene is interrupted by two introns which were identified by comparing the genomic DNA sequence and the cDNA sequence obtained by RT-PCR. The predicted PCO1 protein contains 1101 amino acids and appears to possess a single Zn(II)2/Cys6 binuclear-type zinc cluster. A coiled-coil domain was predicted by computer-aided sequence analysis, suggesting that PCO1 might function as a dimer. A chemical crosslinking assay indicated PCO1 does dimerize *in vitro*. A loss of function *pco-1* mutant was created by the rip procedure. Analysis of *pco1*- strains revealed that PCO1 acts as a positive regulator of the purine degradation pathway. Results of mobility shift assays indicate that PCO1 specifically binds to TCGG-N6-CCGA DNA sequences which exist in promoter regions of the structural genes it regulates. The C-terminus of PCO1 features a domain rich in glutamine, proline, isoleucine and acidic residues which are commonly found in activation domains of transcription factors. This domain shows higher homology to NIT4, the *Neurospora* pathway-specific factor in the nitrate assimilation pathway, than to UAY, its counterpart in *Aspergillus nidulans*, suggesting transcription factors in *N. crassa* may share similar activation regions.

203. Dominant active Rac and dominant negative Rac restore the wild type phenotype of dominant active Ras mutant in *Colletotrichum trifolii*: Involvement with MAP kinase activation and ROS generation. Changbin Chen and Martin B. Dickman, University of Nebraska-Lincoln,U.S.A.

In the filamentous phytopathogenic fungus *Colletotrichum trifolii*, Ras (Ct-Ras) is being studied. Dominant active Ct-Ras(Val2) results in aberrant hyphal morphology, loss of polarity, and inability to differentiate, only under conditions of nutrient deprivation, suggesting the important role of Ras in hyphal growth and development. However, the underlying mechanisms for this response and the relevant pathways regulated by Ras are unclear. This study reports the isolation and molecular characterization of a Rac GTPase gene from *C. trifolii*. Yeast two-hybrid assays and in vitro binding assays indicated that Ct-Rac is a downstream target of Ct-Ras. Expression of dominant active Rac resulted in abnormal hyphal growth and reduced sporulation rate, and expression of dominant negative Rac completely inhibited hyphal growth, suggesting that Rac is important for proper hyphal growth and development. Interestingly, co-expression of dominant active Rac or dominant negative Rac in dominant active Ct-Ras background, both lead to restoration of wild type phenotype. Inhibitor studies and Western analyses demonstrated that MAP kinase activity contributes to phenotypic restoration when dominant active Rac is introduced into the activated Ras strain. In contrast, our results suggest that the decrease of intracellular ROS levels followed by the expression of dominant negative Rac, is necessary for phenotypic restoration of the Ras mutant. Rac appears to mediate two distinct signaling pathways in the regulation of hyphal growth and development in*C. trifolii*; stimulation of MAP kinase and intracellular ROS generation. Our data support a model by which the small GTP-binding protein Rac plays a central role in Ras signaling.

204. The pH- and ROS-regulated MAP kinase signal transduction pathway in *Sclerotinia sclerotiorum*. Changbin Chen and Martin B. Dickman. University of Nebraska-Lincoln, U.S.A.

Sclerotinia sclerotiorum is a ubiquitous phytopathogenic fungus able to infect an extremely wide range of plants. The survival of this fungus is mediated through the sclerotium, a pigmented, multihyphal structure. Molecular mechanisms that trigger and coordinate sclerotial morphogenesis are not well understood. Inhibitor studies suggested involvement of an ERK-like MAP kinase in sclerotial morphogenesis. Therefore we cloned and characterized the ERK-like MAPK homolog in *S. sclerotiorum*. Northern analyses showed that *SSMAPK-1*was highly expressed in low pH but inhibited in high pH conditions. The application of exogenous oxalic acid, a virulence factor in this fungus, induced rapid accumulation of*SSMAPK-1* transcripts, suggesting that *SSMAPK-1* expression in *S. sclerotiorum* is pH-regulated. Moreover, high concentrations of cAMP, which we previously have shown, inhibits sclerotial formation, also suppresses *SSMAPK-1* expression, in a PKA-independent manner. Preliminary experiments using specific toxin inhibitors suggest that Ras or Rap-1 mediates cAMP-induced MAP kinase inhibition. Constitutive overexpression of*SSMAPK-1* bypassed the suppressive effect of cAMP and induced sclerotial development. Similar to ERK inhibitor (PD98059) experiments, expression of antisense*SSMAPK-1* inhibited sclerotial maturation. We further show the involvement of reactive oxygen species (ROS) generation in sclerotial development. The application of exogenous H_20_2 induced *SSMAPK-1* expression and high concentrations of antioxidants such as NAC and DPI inhibited sclerotial maturation and*SSMAPK-1* expression, suggesting ROS-

dependent induction of the MAP kinase pathway possibly accounts for sclerotial morphogenesis. Thus, our findings demonstrate that*SSMAPK-1* is pH- and ROS- regulated and is required for sclerotial development.

205. The G-protein beta subunit is required for appressorium formation and conidiation in *Magnaporthe grisea*. Marie Nishimura[1] and Jin-Rong Xu[2]. [1]National Institute of Agrobiological Sciences, Tsukuba, Japan. [2]Purdue University, West Lafayette, IN, USA.

Heterotrimeric G-proteins transmit extracellular signals to various downstream effectors in eukaryote. In the rice blast fungus *Magnaporthe grisea*, appressorium formation is induced by surface hydrophobicity or chemical inducers such as cAMP. Pmk1, a MAP kinase homologous to yeast Fus3/Kss1, is known to regulate appressorium formation and infectious hyphal growth. Because gene disruption mutants of three G-protein alpha subunits exhibit phenotypes different from that of *pmk1* mutants, in this study we isolated and characterized the function of the G-protein beta subunit gene (*MGB1*) in *M. grisea*. MGB1 is a single copy gene in M. grisea and it is highly homologous to the G-beta subunits identified in *Cryphonectria parasitica* and other filamentous fungi. Gene disrupted mutant of *MGB1* showed abundant mycelial growth, but was reduced in conidiation. Conidia from *mgb1* mutants were defective in appressorium formation on hydrophobic surface and failed to form lesions on rice plants. With the presence of exogenous cAMP, *mgb1*mutants formed abnormally-shaped appressoria on hydrophobic or hydrophilic surfaces. However, these appressoria induced by cAMP were not functional and failed to penetrate and develop infectious hyphae in plant cells. In mycelia and spores harvested from one week old oatmeal cultures, the cellular cAMP concentration in *mgb1* mutants was reduced to 60% of the wild type. Interestingly, conidia collected from 7-10 day old cultures of transformants carrying multi-copy of *MGB1* (>2 copies) could form appressoria on hydrophilic surface (non-inductive condition). Cellular cAMP concentrations in these transformants were 60% higher than that of the wild type strain Guy11. These data suggest that *MGB1* is involved in regulating appressorium formation, plant infection, and conidiation, likely by controlling the adenylate cyclase activity.

206. Activation of *chsC* transcription by AbaA during asexual development of *Aspergillus nidulans*. Bum-Chan Park, Yun-Hee Park and Hee-Moon Park. Department of Microbiology, College of Natural Sciences, Chungnam National University, Daejeon 305-764, Korea

The temporal and spatial regulation of chitin synthesis plays an important role in morphogenesis during fungal growth and development. Northern blot analysis showed that the transcription level of *chsA*, *chsC*, and *chsD* was significantly decreased in an *Aspergillus nidulans abaA* mutant. Electrophoretic mobility shift assays revealed that AbaA bound tightly to all three AREs (AbaA response elements) in the *chsC* promoter region. Experiments with the *Saccharomyces cerevisiae* heterologous expression system confirmed AbaA-dependent transcriptional activation of *chsC*. Taken together, these data suggest that AbaA plays an important role in chitin biosynthesis during conidiophore development by controlling the transcription level of certain chitin synthase genes. (This work was supported by a grant form KOSEF, Project No. R01-1998-000-00053-2001.)

207. Pka, Ras and RGS Protein Interactions Regulate Sterigmatocystin Biosynthesis in*Aspergillus nidulans*. Kiminori Shimizu[1], Julie K. Hicks[2,3], Tzu-Pi Huang and Nancy P. Keller. Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706 and [2]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843. Present address: [1]Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-8673, Japan,[3]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710

Sterigmatocystin (ST) is a carcinogenic polyketide produced by several filamentous fungi including *Aspergillus nidulans*. Expression of ST biosynthetic genes (*stc* genes) requires activity of a Zn(II)2Cys6 transcription factor, AflR. *aflR* is transcriptionally and post-transcriptionally regulated by a G-protein/cAMP/protein kinase A (PkaA) signaling pathway involving FlbA, a RGS (Regulator of G-protein Signaling) protein. Prior genetic data showed that FlbA transcriptional regulation of *aflR* was PkaA dependent. Here we show that mutation of three PkaA phosphorylation sites in AflR allows resumption of *stc*expression in an overexpression *pkaA* background but does not remediate stc expression in a DflbA background. This demonstrates negative regulation of AflR activity by phosphorylation and shows that FlbA post-transcriptional regulation of *aflR* is PkaA independent. AflR nucleocytoplasmic location further supports PkaA independent regulation of AflR by FlbA. GFP tagged AflR is

localized to the cytoplasm when *pkaA* is overexpressed but nuclearly located in a DflbA background. Genetic interaction between AflR and RasA is also investigated.

208. Comparative promotor analysis of four polygalacturonase genes of *Fusarium oxysporum* f.sp. *radicis lycopersici* in *Saccharomyces cerevisiae.* González-Jaén, M.T., A. de las Heras, B. Patiño, M.J. Bueno, C. Vázquez. Department of Genetics, Univ. Complutense of Madrid, Spain.

Fusarium oxysporum f.sp. *radicis lycopersici* causes crown and root rot disease in tomato plants, a disease characterized by extensive cell wall degradation. Four polygalacturonase (PG) coding genes have been identified so far which corresponded to two EXOPG and two ENDOPG coding genes regulated at transcriptional level in response to different metabolic inductors in in vitro cultures. The differences observed in their patterns of gene expression suggested the presence of some differences in the regulatory motifs present in their promotor regions. We report the putative regulatory motifs identified in the sequence of the promotor region of the four pg genes and their analysis in a yeast system in response to different conditions related with carbon source and pH. The results support the differential regulation of those genes and the significance of regulation in infection and symptom development. The system of promotor analysis used in this work offers a high potential for the analysis of other Fusarium genes.

209. Polyketide synthases and non-ribosomal peptide synthetases in *Neurospora crassa*. Nabil Arrach, Scott Kroken and Louise Glass, Department of Plant and Microbial Biology, University of California, Berkeley

The type I polyketides (PKs) and non-ribosomal peptides (NRPs) constitute one of the most diverse groups of natural products, and are common in bacteria and in fungi. The characterized metabolites often function as toxins against hosts and competitors, targeting various aspects of metabolism. In ascomycete fungi, most PKs and NRPs have been characterized from plant pathogens, in which they often serve as virulence factors needed for pathogenicity against their host plants, and from ecologically competitive saprobes such as *Aspergillus flavus*, with which they fend their food supplies against invaders. Surprisingly, *Neurospora crassa* contains at the least 7 PK synthases and 3 NRP synthetases, even though it is not known to make any secondary metabolites which function as toxins. In the eucaryotic model Dictyostelium, a diffusible signal molecule called DIF-1 induces the differentiation of prestalk-O cells. DIF-1 is a chlorinated alkyl phenone that is synthesized from a C12 polyketide precursor (Thompson CR and Kay RR., 2000). In bacteria, some compounds that are involved in toxins in higher concentration may act as a signalling compounds in low concentration. For example, it has recently been shown that aerial hyphae formation appears to be especially sensitive to inhibition by protein kinase inhibitors in Streptomyces (Waters et al., 2002). Our hypothesis is that polyketides and non-ribosomal peptides in Neurospora could play a role in a developmental aspect such as conidiation, hyphal fusion or mating.

210. Effect of G-protein signalling in *Fusarium culmorum*. Jakob Skov and Henriette Giese. Section of Genetics and Microbiology, Department of Ecology The Royal Veterinary and Agricultural University Thorvaldsensvej 40, DK-1871 Copenhagen, Denmark

In filamentous fungi G-proteins are involved in regulation of a wide range of processes including development, pathogenicity and secondary metabolism. A G-protein alfa-subunit gene,*fadA*, was previously isolated from *Aspergillus nidulans*. The protein has been shown to affect secondary metabolism in *A. nidulans* as well as in the plant pathogen *Fusarium sporotrichioides* (Tag *et al*. 2000, *Mol. Microbiol*.**38**(3):658-665). *F. culmorum* is the predominant *Fusarium* species in Danish soils and is believed to be the major cause of Fusarium Head Blight of cereals in Denmark. *F. culmorum* is known to produce an array of mycotoxins. In this study *F. culmorum* was transformed with a construct containing a constitutively signalling allele of *fadA* using the *Agrobacterium*-mediated transformation system. Integration was tested by Southern analysis and expression of the transgene was analysed using RT-PCR. The effect of the gene on secondary metabolism was examined using HPLC. To detect yet unknown downstream targets of the G-protein signalling pathway the protein profile of transformants was compared to wildtype using 2D electrophoresis.

211. Characterization of a mutant defective in the Neurospora *ncMMS2* gene, a homologue of *Saccharomyces cerevisiae MMS2*. Tsuyoshi Kawabata, Keiichiro Suzuki, Hirokazu Inoue. Laboratory of Genetics, Department of Regulation Biology, Faculty of Science, Saitama University, Saitama City 338-8570, Japan.

MMS2 and *UBC13* of *S. cerevisiae* code a ubiquitin conjugating enzyme variants (UEVs). Products of these two genes form a heterodimer and act in post replication repair (PRR). The heterodimer functions in error-free PRR. Mutants of these genes showed higher sensitivity to UV and MMS than the wild type. We have made a mutant of *ncMMS2* gene in *Neurospora crassa* and investigated its role in DNA repair. The mutant was sensitive to MMS like yeast *mms2*, however, not sensitive to UV. The *ncMMS2* cDNA sequence was determined and deduced aminoacid sequence was compared with those of yeast and human. We made double mutants of*ncMMS2* and other DNA repair genes, in order to analyze epistatic relationship. Mutation frequency at the *ncMMS2* mutant are also presented.

212. ACEI is a repressor of cellulase and xylanase genes in *Trichoderma reesei*. Aro, N., Ilmén, M., Saloheimo, A., Penttilä, M. Biotechnology. VTT. Espoo, Finland

T. reesei is an efficient producer of cellulolytic and xylanolytic enzymes. In general, cellulose and its derivatives and different xylans induce cellulase and xylanase expression while glucose represses cellulase and xylanase genes. The production of these enzymes is regulated at the transcriptional level at least by the glucose repressor CREI and the activator ACEII. We have isolated a third factor, ACEI, that binds to and activates the main cellulase promoter *cbh1*in vivo in yeast. ACEI is a Cys_2-His_2 type of a transcription factor that binds in vitro to eight sites scattered along the *cbh1* promoter containing the core 5'AGGCA sequence. Although originally isolated as a protein capable of activating the*cbh1* promoter of *T. reesei* in *S. cerevisiae*, further studies of*ace1* deletion strain indicated that ACEI is a repressor of cellulase and xylanase genes. The deletion of the *ace1* gene led up to 10 times higher expression of cellulase genes, *cbh1, cbh2, egl1*, and *egl2* after the transfer of glycerol grown mycelia to cellulose media or when the cellulase genes were induced by the addition of a disaccharide sophorose into glycerol media. Similarly, the expression of *xyn1* and *xyn2* was increased by *ace1* deletion. Deletion of the *ace2* gene, encoding the ACEII activator, in a strain deleted for *ace1* did not affect the high level of cellulase expression seen in the *ace1* deletion strain indicating, that there is at least one additional cellulase and xylanase activator present in *T. reesei*.

213. Sugar sensing and regulation of carbon transport and physiology in Neurospora. Xin Xie, Alejandro Correa, Zach Lewis, Deborah Bell-Pedersen and Daniel J. Ebbole Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA

Sensing of carbon sources in the environment is important for appropriate regulation of gene expression for carbon utilization and as a behavioral cue. In *Saccharomyces cerevisiae*, several signal transduction pathways have been demonstrated in glucose sensing and gene regulation. Two glucose transporter homologs, Snf3 and Rgt2, play important roles in the sensing mechanism. An apparent counterpart in Neurospora is rco-3. rco-3 mutants have altered regulation of sugar uptake, carbon catabolite repression, and conidiation. A second Neurospora gene, dgr-1, displays a phenotype that is very similar to rco-3. Suppressors of the rco-3 and dgr-1 mutants have been used to distinguish the roles of the rco-3 and dgr-1 genes. In addition, transcriptional profiling of the response of Neurospora to glucose starvation have been performed to help define the extent to which glucose availability globally regulates gene expression in a filamentous fungus. Further transcriptional profiling studies in rco-3 and dgr-1 mutants reveals the extent to which cells are defective in glucose sensing and the effect of these mutants on cellular physiology and development.

214. Analysis of the MAK-2 MAP kinase pathway in *Neurospora crassa* identifies secondary metabolism as a target for the mating pathway of filamentous fungi. Dan Li, Piotr Bobrowicz, and Daniel J. Ebbole. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA

MAP kinases in filamentous fungi homologous to the yeast FUS3/KSS1 MAP kinases of *Saccharomyces cerevisisae* have been shown to be important for plant pathogenesis. Characterization of the downstream targets of the pathway and characterization of the role of the MAP kinase in mating is greatly facilitated by use of a model organism, such as Neurospora. The genes for MAP kinase MAK-2 and the downstream transcription factor homologous to Ste12p have been cloned and mutated. Both mutants have growth defects, have reduced aerial growth but normal conidiation, and fail to form protoperithcia but function as males in sexual crosses. However, ascospores of mak-2 and ste-12 null mutants are inviable. Initial analysis has identified classes of genes that are regulated by mak-2 and/or ste-12. A gene cluster containing a polyketide synthetase is one target of MAP kinase regulation. The ‘mating' MAP kinase pathway in filamentous fungi controls many functions other than mating, and the identification

of these functions, such as regulation of secondary metabolite production, may be relevant to understanding the basis for the evolution of plant-fungus interactions.

215. FL, the major regulator of conidiation in *Neurospora crassa*, binds to the promoter of the developmentally-regulated hydrophobin gene, *eas*. Panan Rerngsamran, and Daniel J. Ebbole. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA

The fluffy gene of *N. crassa* is required for the switch from filamentous to budding growth during conidiophore morphogenesis. The FL protein is an 88-kDa polypeptide containing a typical fungal Zn2Cys6-DNA binding motif. As a step towards identifying the target genes on which FL may act, we sought to identify target sequences to which the FL protein binds. The DNA binding domain of FL was expressed in *Escherichia coli* as a fusion with glutathione S-transferase (GST) and purified using glutathione sepharose affinity column. The DNA-binding sites were selected and amplified by means of a PCR-mediated random-site selection method involving affinity bead-binding and gel-mobility shift analysis. Sequencing and comparison of the selected clones suggested that FL binds to the motif 5'-CGGN9CCG-3'. The potential binding site was found in the promoter region of the eas (ccg-2) gene, which encodes a fungal hydrophobin. In vitro competitive binding assays revealed the preferred binding site for FL in eas promoter is 5'-CGGAAGTTTCCTCCG-3'which is located nearly 1500-bp upstream of the eas transcription initiation site. In vivo experiment using a foreign DNA sequence tag also confirmed that this sequence is a target site for FL regulation. Additionally, in vivo experiments confirm that transcriptional activation activity resides in the C-terminal half of FL.

216. Expression and sequence analysis of a *Ste 20* homologue from the phytopathogenic fungus *Fusarium oxysporum*. M A. García-Sánchez, B. Ramos, A. P. Eslava and J.M. Díaz-Mínguez. Area de Genética, Centro Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca. 37007, Salamanca, Spain

Fusarium oxysporum is a deuteromycetous fungus and a significant plant vascular pathogen with worldwide distribution. Studies on the signal transduction MAPK cascade that operate in fission yeast, human pathogens, plant pathogens and model filamentous fungi reveal a high degree of conservation and the involvement of this pathway in fungal development, mating and pathogenicity. Genetic analysis conducted in several plant pathogenic fungi have demostrated that the MAPK signal transduction pathway plays an important role in pathogenesis A key link of the MAPK cascade with upstream elements seems to be the STE20 protein. STE20 is a serine-threonine protein kinase shown to be activated in vitro by GTP-bound Cdc42. Previously, we reported the cloning of a fragment of a *Ste20* homologue from the plant pathogenic fungus *F. oxysporum*, by means of PCR using primers based on conserved amino acids kinase domains. Here we report the cloning of a full genomic copy of this gene and the molecular analysis of the sequence of this gene. Also, we have analyzed the expression of this gene in vitro by RT-PCR of RNA samples from in vitro grown fungal cultures, and in vivo by real-time PCR using RNA from bean plants collected at different times of the infection process. Gene disruption experiments are being developed so as to functionally characterize the role of this gene in pathogenicity and/or virulence. This research was supported by grant AGL 2001-2052 (Ministerio de Ciencia y Tecnología of Spain). M.Asunción García-Sánchez was the recipient of a fellowship from Caja Rural de Salamanca (Castilla y León, Spain) and Brisa Ramos was the recipient of a fellowship from the INIA (Ministerio de Ciencia y Tecnología of Spain).

217. The winged helix/RFX transcription factor CPCR1 affects both cephalosporin C gene expression and hyphae morphogenesis in *Acremonium chrysogenum*. B. Hoff, E. Schmitt, D. Janus and U. Kueck. Dept. of General & Molecular Botany, Ruhr-University Bochum, Universitätsstr. 150, 44780 Bochum, Germany, Fax: +49 234 3214184

Acremonium chrysogenum is the industrial main producer of the beta-lactam antibiotic cephalosporin C (CPC). The expression of the corresponding biosynthesis genes is mostly regulated on the transcriptional level. Using the one-hybrid system we have isolated the transcription factor CPCR1 which binds to an imperfect palindromic sequence in the intergenic promotor region of the *pcbAB/pcbC* genes and belongs to the conserved family of eukaryotic RFX transcription factors [1]. Here we provide further data of the functional analysis of the transcription factor CPCR1. To determine the effect of CPCR1 on transcription of the *pcbC* gene, two approaches were followed. On the one hand we have constructed strains with multiple copies of the *cpcR1* gene in order to increase gene expression. On the other hand we have generated fungal strains with a disrupted *cpcR1* gene. All transformants were investigated

using northern and western analysis to study changes in the CPC gene expression. These data shows an increased*pcbc* gene expression in the multi copy strains, while the opposite effect is observed in the knock-out strain. Most interestingly, the alteration of the copy number of the *cpcR1* gene in the genomic DNA of fungal transformants does not only influence CPC biosynthesis gene expression but has also major effects on hyphal morphology and differentiation. Growth curves as well as light and fluorescence microscopic studies using GFP labelled protein revealed differences in biomass accumulation and hyphal morphologies in the transformed strains. The time course of arthrospore formation, that is the organized fragmentation of hyphae, is accelerated in multicopy *cpcR1* strains during batch cultivation. Our data indicate a dual function of the CPCR1 transcription factor in CPC gene expression as well as in arthrospore formation.

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218. Mutation in a Calpain-like Protease Affects the Posttranslational Mannosylation of Phosphatases in *Aspergillus nidulans*. Sérgio R. Nozawa [(1)], Gregory S. May [(2)], Monica S. Ferreira-Nozawa [(1)], Nilce M. Martinez-Rossi [(1)] and Antonio Rossi [(3)]. [(1)] Depto. de Genética, FMRP-USP, Ribeirão Preto, SP, Brazil; [(2)] Division of Pathology, M.D. Anderson Cancer Center, Houston, TX, USA; [(3)] Depto. de Bioquímica e Imunologia, FMRP-USP, Ribeirão Preto, SP, Brazil.

The ambient pH responses are mediated in *A. nidulans* by a conserved signal transduction pathway comprising at least seven genes (*pacC* and *palA*, etc) that have been cloned and sequenced. The *palB* gene codes for a calpain-like protease that is not involved directly in PacC processing, and the other *pal* genes have revealed only few functional features. In this communication, we show that the *palB7* mutation drastically reduced the mannose and N-acetylgalactosamine content of the *pacA*-encoded acid phosphatase secreted by *A. nidulans* at pH 5.0, compared to a control strain. By using mRNA differential display reverse transcription and polymerase chain reaction, we isolated two cDNAs from the control strain (*pabaA1*) that were not detected in the *palB7*mutant strain and that encode a mannosyl transferase and a NADH-ubiquinone oxidoreductase. Thus, a defect in the posttranslational mannosylation of proteins could be the consequence of mutations in the *palB* gene, which codes for a nuclear calpain-like protease that may have specific functions in the processing of transcription factor(s) like its homologue, *RIM13*, in *S. cereviseae*. Further evidence in this direction comes from the demonstration that mutations in the mammalian calpain 3 protease rather than a structural defect can cause limb-girdle muscular dystrophy type 2A, and that these mutations are pathogenic only in a specific mitochondrial context (Richard *et al*., Cell, **81**, 27-40, 1995). Thus, a defect in the posttranslational mannosylation of proteins could be the consequence of mutations in the proteolytic enzyme calpain 3 and could promote muscular dystrophy type 2A in humans. Financial support: FAPESP, CNPq, CAPES, FAEPA and Reitoria-USP.

219. Disruption of Gene *Pac*c Affected Both Growth and Conidiation of the Dermatophyte *Trichophyton rubrum*. Ferreira-Nozawa, M.S. [(1)], Nozawa, S.R [(1)], Cervelatti, E.P.[(1)], Rossi, A. [(2)] and Martinez-Rossi, N.M. [(1)].[(1)] Depto de Genética, FMRP-USP, Ribeirão Preto, SP, Brazil.[(2)] Depto de Bioquímica e Imunologia, FMRP-USP, Ribeirão Preto, SP, Brazil.

Although it has been well established that ambient pH affects the growth, physiology, differentiation, and viability of all organisms, the molecular responses to environmental pH changes are only now being elucidated. In *Aspergillus nidulans* and other fungi these responses are mediated by gene *pac*C, which codes for a Zn-finger transcription factor that activates the expression of several genes at alkaline pH. The *pac*C transcription is itself induced under alkaline growth conditions. In order to investigate the role of the*pac*C gene in the adaptive response of the dermatophyte *Trichophyton rubrum* to ambient pH, an important event in the dermatophyte-host interactions, we have cloned and identified a gene of this fungus homologous to the *pac*C gene of *A. nidulans* and *pac*C-1 of *N. crassa* (GenBank-access number: AF363788). The northern and western blots of *T. rubrum* also showed an increase of *pac*C transcripts at alkaline pH. For disruption, the promoter and first zinc-finger (3.5 Kb) were removed in the genomic clone of 6.0 Kb and replaced with the hygromycin phosphotransferase gene (*hph*), which confers drug resistance on eukaryotic cells. Several transformants of *T. rubrum* were isolated and showed that growth, conidiation, and pigmentation of the colonies were affected by the disruption of gene *pac*C, as already observed for *C. albicans* and *N. crassa*, indicating that surviving a proton attack depends on the expression of genes (*e.g. pac*C gene) that determine sensitivity or resistance to pH. Furthermore, these mechanisms are apparently involved in the

installation, development, and survival of dermathophytes in humans. Financial support: FAPESP, CNPq, CAPES, FAEPA and Reitoria-USP.

220. The *atrE* gene of *Trichophyton rubrum* is responsive to cyclohexamide, griseofulvin, azole antifungal agents and ethidium bromide. Cervelatti, E.P.; Fachin, A.L.; Ferreira-Nozawa, M.S. and Martinez-Rossi, N.M. Departamento de Genética, FMRP-USP, Ribeirão Preto, SP, Brazil.

Trichophyton rubrum is the most commonly species found in superficial lesions of the skin, nails and hair. Over the last few years, the improper use of antifungal agents, the practice of invasive medical techniques and the onset of AIDS have led to cases of re-incidence of mycoses caused by this pathogen, as well as its invasive behavior. Despite the importance of this dermatophyte, very little is known about its genetic and biological aspects. As a contribution to this knowledge the goal of the present study was to characterize a gene coding for a carrier of the ABC type, belonging to a family of proteins involved in multidrug resistance mechanism. To this end, a 230 bp probe obtained by PCR presenting high identity with ABC carriers described in other organisms was used to track the complete gene in a genomic library. The sequence of the identified clone demonstrated a 5243 bp gene (GenBank-access number AF525740) homologous to the *atrE* gene of *Aspergillus nidulans*. To analyze the functionality of the identified gene, the fungus was cultured in the presence of various cytotoxic agents for 15, 30 and 60 minutes. The total RNA was used in a northern blot experiment and the expression of the gene of interest was evaluated using a central region of the gene as a molecular probe. An increase gene expression of the *atrE* transcript was observed after 30 minutes of exposure to ethidium bromide, ketoconazole, cyclohexamide, fluconazole, griseofulvin and itraconazole, suggesting the participation of the identified gene in drug efflux and its potential use as a therapeutic target for drugs to be developed by the pharmaceutical industry.

Financial support: FAEPA, Reitoria-USP.

221. Regulation of Neurospora fatty acid desaturase expression. Pitchaimani Kandasamy, Chan-Seok Oh, Ramesh Chellappa, Seung-Jae Baek and Charles E. Martin. Rutgers University, Division of Life Sciences, Nelson Laboratories, 604 Allison Road, Piscataway, NJ 08854.

Unsaturated fatty acids are formed by a series of membrane bound enzymes that form double bonds in long (C_{14} – C_{18}) chain species. *N. crassa* encodes at least three desaturases that form the major membrane lipid fatty acids: Delta-9 18:1, Delta 9, 12 18:2 and Delta 9,12,15 18:3. Our studies on the *OLE1* gene, which encodes the only long chain desaturase in yeast (a Delta-9 enzyme), demonstrated that its expression is regulated at the levels of transcription and mRNA stability by fatty acids and molecular oxygen.*OLE1* is also transiently regulated in response to shifts to low temperature. Our previous studies also showed that the Neurospora Delta-15 desaturase is strongly regulated by growth temperature and recent experiments show that expression of the Neurospora desaturases is also regulated by unsaturated fatty acids. Expression of the yeast desaturase is governed by membrane bound proteins that are essential for transcription activation and hypoxic induction of*OLE1*. These activate *OLE1/* expression through their N-terminal domains which are released from the membrane through an ubiquitin-mediated mechanism that involves processing by the 26S proteosome. The ectopic integration of the homologous Neurospora genes that encode these proteins appear to quell growth. This can be repaired by unsaturated fatty acids, suggesting that a similar mechanism may be common to other fungi. (supported by NIH grant GM45768)

222. ER stress response: The *A. niger* transcription factor HacA mediates the upregulation of ER target genes, *bipA, cypB and pdiA,* and its own gene. Harm J. Mulder, Igor V. Nikolaev, and Susan M. Madrid Danisco Innovation Copenhagen, Langebrogade 1, DK 1001, Copenhagen, Denmark.

In eukaryotic cells, accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum (ER) constitutes a fundamental threat to the cells. To deal with this stress, eukaryotic cells can respond to it via three different mechanisms: Transcriptional induction, translational attenuation, and degradation. When unfolded proteins accumulate in the ER the cell reacts by upregulating the synthesis of ER resident protein-chaparones and foldases, thereby increasing the folding capacity in the ER. The response, which involves a signal transduction cascade from the ER to the nucleus, is often referred to as the Unfolded Protein Response (UPR). The mRNA encoding the transcriptional activator HacA undergoes unconventional splicing upon accumulation of unfolded proteins within

the ER, resulting in efficient translation of hacA, and activation of the UPR. Northern analysis of Aspergillus strains overproducing *hacA*, showed in addition to the upregulation of *bipA, cypB and pdiA*, also an increase in the amount unspliced mRNA form of *hacA*, which could indicate that the HacA protein also is involved in the upregulation of its own gene under UPR conditions. DNA binding experiments showed indeed binding of HacA protein to a *hacA* promoter fragment, which strengthen this hypothesis.

223. Multiple roles of WHITE COLLAR-1 in the environmental-sensing signal transduction pathways. Kwangwon Lee, Jennifer J. Loros, and Jay C. Dunlap, Department of Genetics and Department of Biochemistry, Dartmouth Medical School. Hanover NH. 03755.

The transcription factors WHITE COLLAR-1 (WC-1) and WHITE COLLAR-2 (WC-2) interact to form a heterodimeric complex (WCC) that is essential for most light-mediated processes in *Neurospora crassa*. WCC also plays a distinct non-light-related role as the transcriptional activator in the FREQUENCY (FRQ)/WCC feedback loop that is central to the*N. crassa* circadian system. Although an activator role was expected for WC-1, unanticipated phenotypes among some *wc-1* alleles prompted a closer examination of an allelic series for WC-1 that has uncovered roles for this central regulator in constant darkness and in response to light. Using microarray technology we investigated the roles of WC-1 in general environmental-sensing signal transduction pathways including, light, temperature and nutrition-starvation. We discovered novel regulatory mechanisms of light-regulation. Our data suggest that 1) WC-1 is not only a positive regulator but also a negative regulator, 2) there are light-receptors other than WC-1, and 3) there is possible hierarchical transcriptional regulation among light-receptors. Our data also suggest that WC-1 has regulatory roles for subsets of stress-regulated genes, likely through indirect mechanisms. Supported by NIH (GM20553 to KL, GM34985 to JCD) and NSF (0084509 to JJL).

224. The role of chromatin mediated transcriptional regulation during pathogenic development in *Ustilago maydis.* Alexander Jamnischek, Martina Treutlein, Mario Scherer, and Joerg Kaemper, MPI for terrestrial Microbiology, Marburg, Germany

In the phytopathogenic fungus *Ustilago maydis*, pathogenic development is controlled by the two homeodomain proteins bE and bW encoded by the *b* mating type locus. The bE/ bW heterodimer is thought to control genes involved in pathogenicity as a transcriptional regulator either directly or indirectly via a *b*-mediated regulatory cascade. We have recently identified two genes, *hda1* and *rum1*, that both play a role in the repression of *b*-regulated genes in the absence of a bE/bW heterodimer. The *hda1* gene encodes a histone deacetylase (HDAC), and for Rum1 the domain structure suggests, similar to Hda1, a role in chromatin mediated transcriptional regulation. The deletion of either gene leads to a discrete block during teliospore development. Our current model places both protein in a common complex that regulates a defined set of genes by chromatin modification. By in silico analysis we have now identified a second HDAC, Hda139. In contrast to Hda1 that appears to be involved in the repression of *b*-regulated genes, Hda139 seems to be required for their activation after formation of an bE/bW heterodimer. *hda139* mutant strains show reduced growth, defects in polar growth and filament formation, and are apathogenic. We will present results of a genome wide expression analysis of 6300 *U. maydis* genes that allow to address the distinct pathways affected in the *rum1*,*hda1* and *hda139* deletion strains.

225. *AzoA*, a novel molecular determinant of sensitivity to azoles in *Aspergillus nidulans.* Alan C. Andrade[1], Luc Rouws[2] and Maarten A. De Waard[2]. [1]Lab. de Gen. e Biol. Molecular, EMBRAPA Recursos Genéticos e Biotecnologia, Caixa Postal 02372, Brasília-DF, Cep: 70770-900, Brazil.[2]Lab. of Phytopathology, Department of Plant Sciences, Wageningen University,P.O.Box 8025, Wageningen, 6700-EE, The Netherlands

Azoles constitute an important class of antifungal agents widely used in both agriculture and clinical medicine. Their efficacy is often limited by a variety of biological processes. Among these, the overexpression of membrane efflux pumps of the ABC (ATP-binding cassette) and MF (major facilitator) families, which lower intracellular drug concentration below effective levels, is a major but complex mechanism that results in multidrug resistance (MDR). MDR is considered to be the major challenge for modern chemotherapy. Here we describe the cloning and characterization of *azoA*, which is a novel molecular determinant of sensitivity to azoles in *Aspergillus nidulans*. This gene was identified from an azole-hypersensitive mutant of *A. nidulans*, obtained by insertion mutagenesis followed by plasmid rescue. Blast analysis of AzoA revealed similarity to hypothetical proteins with unknown function from yeasts and other fungi. Northern analysis revealed increased transcript levels of *azoA* upon treatment

of fungal germlings with azoles. Furthermore, gene-replacement mutants of *azoA*, also displayed increased sensitivity to azoles, confirming its functional role. Interestingly, transcript levels of *atrG*, a previously characterized ABC transporter involved in protection of *A. nidulans* against azoles is higher in *delta azoA* mutants. Additionally, all *delta azoA* mutants displayed lower accumulation of [^{14}C]fenarimol, as compared to a control strain. This situation mimics MDR, e.g reduced intracellular accumulation due to overexpression of an efflux pump (AtrG). Nevertheless, the phenotype observed for *delta azoA* mutants is increased sensitivity to azoles. These results suggest a major role for *azoA* on fungal sensitivity to azoles.

226. Microarray Analysis of Vegetative Incompatibility in *Neurospora crassa*. Sarah C. Brown, Isao Kaneko, Takao Kasuga, John W. Taylor and N. Louise Glass Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720

Neurospora crassa, like other filamentous fungi, is capable of spontaneous hyphal fusion during vegetative growth. Hyphal fusion between genetically distinct individuals leads to the formation of heterokaryons. The viability of such heterokaryons is dependent on the alleles present at loci for heterokaryon incompatibility (*het* loci). If hyphal fusion occurs between individuals with different alleles at a given *het* locus, hyphal compartmentation and ultimately cell death occur. Cell death observed during vegetative incompatibility bears several of the hallmarks of programmed cell death, including vacuolization of the cytoplasm, organelle degradation and shrinkage of the plasma membrane from the cell wall. We are investigating cell death during vegetative incompatibility using a temperature sensitive mutant that is able to form stable heterokaryons with a normally incompatible partner at 34°C. Vegetative incompatibility initiates after transfer to 20°C. Oligonucleotide microarrays have been developed and hybridization conditions optimized to compare gene expression patterns under different growth conditions. We are using these arrays to analyze gene expression in the temperature sensitive mutant at both 34°C and 20°C. We aim to identify genes that play an active role in the control of cell death in vegetative incompatibility in *N. crassa*.

227. Mapping the limits of the *TRI4*/*TRI6* bidirectional promoter in*Fusarium graminearum* and analysis of its transcription factor binding sites. Rex B. Dyer, David Kendra, and Daren W. Brown. Mycotoxin Research Unit, USDA/ARS/NCAUR, Peoria, IL 61604

Fusarium graminearum, the causative agent of wheat scab, is an international problem due to economic losses to the agricultural industry and because of food safety concerns resulting from mycotoxin contamination. Mycotoxin synthesis is coordinated by the*TRI6* gene which encodes a transcription factor. In order to better understand the regulation of *TRI6* gene expression and mycotoxin synthesis, we have employed the method of 5'-RACE to map the limits of the bidirectional promoter within the*TRI4/TRI6* intergenic region of *F. graminearum*. We conclude that this bidirectional promoter consists of 892 nucleotides, mapping from -109 to -1000 nucleotides 5' of the *TRI4* translational start codon. We analyzed the DNA sequence of this bidirectional promoter and the analogous sequence from 41 other *Fusarium* strains for transcription factor binding sites. Our analysis of the 42 strains reveals 21 to 30 transcription factor binding sites, specific to 14 different transcription factors. The transcription factor binding site that is most commonly represented in each strain is specific to the *NIT2* transcription factor and is involved in nitrogen metabolite repression. We hypothesize that the *NIT2* sites within the *F. graminearum TRI4*/*TRI6* bidirectional promoter function to bring *TRI4* and *TRI6* gene expression, and mycotoxin biosynthesis under nitrogen metabolite repression control.

228. Characterization and Cloning of *arg-13* mutants from *N. crassa*. Gloria E. Turner, Giselle Galang & Richard L. Weiss. Department of Chemistry, UCLA, California

The role of *Neurospora crassa arg-13* gene product in arginine biosynthesis is not understood. The cloned gene sequence revealed homology with the mitochondrial carrier family (MCF) proteins. Most MCF proteins are localized in the inner mitchondrial membrane and participate in metabolic trafficking, exchanging metabolites for inorganic cations. This is accomplished using dual transport mechanisms, uniport and exchange. Examples include the dicarboxylate carrier, citrate carrier and the ornithine, glutamate and glutamine carriers. To understand the role of ARG13 in *N. crassa* arginine metabolism we have characterized four *arg-13* mutant alleles. The mutants have a slow growth phenotype which can be restored to normal growth by the addition of arginine, citrulline and to a lesser extent ornithine. The ability of ornithine to support normal growth is problematic for one allelle. This result suggests that ARG13 may be exchanging ornithine and the need for arginine is a result of unregulated ornithine movement

out of the mitochondrion. We have constructed the mutant strain *aga arg-13 ota* to test this hypothesis. Additionally we have cloned and sequenced two *arg-13* mutant alleles using PCR amplification. A comparison of the mutant, wildtype and other known MCF proteins will enable us to determine important residues for ARG13 function. An *arg-13* deletion has been constructed and will be used for *in vivo* analysis of altered ARG 13 proteins.

229. The conserved zinc finger domain of *Aspergillus nidulans* SteAp regulates the frequency of cleistothecial initial formation in a *veA1* background. Emily J. Telfer and Bruce L. Miller. MMBB, Universtity of Idaho, Moscow, ID.

The SteAp of *A. nidulans* contains an N-terminal homeodomain, homologous to the*S.cerevisiae* Ste12p homeodomain, as well as two C-terminal C_2/H_2 Zn^{+2} fingers, conserved in Sterile 12-like proteins of filamentous fungi and several dimorphic fungi. Sexual development was examined in an *A. nidulans veA1* strain carrying an in frame deletion of the *steA*C_2/H_2 Zn^{+2} finger domain. Consistent with results seen in the *steA* disruption mutant, the *steA*C_2/H_2 Zn^{+2} finger deletion mutant (*steA*:delta ZnF) does not affect conidiation. However, in contrast to the *steA*disruption mutant, steA:delta ZnF produces fertile cleistothecia, containing wild type numbers of viable ascospores (1,000 - 100,000). *steA*:delta ZnF also suppresses the *veA1* mutation with respect to sexual fertility and increases not only the frequency, but also the consistency with which sexual development is initiated. These results suggest *steA* interacts genetically with *veA* to regulate the frequency with which cleistothecia are initiated. The C_2/H_2 Zn^{+2} finger domain, which has not been shown to bind DNA, may provide a mechanism for this regulation.

230. Cloning of trehalose 6-phosphate phosphatase gene, *orlA*, from *Aspergillus nidulans* and analysis of its expression in response to stress and development. Jeong Heon Lee[1], Mo Young Yu[1], Je Seop Park[1], Jin Young Jeon[1], Dong Min Han[2], and Pil Jae Maeng[1]. [1]Department of Microbiology, Chungnam National University, Daejeon 305-764; and [2] Division of Life Sciences, Wonkwang University, Iksan 570-749, Korea.

Aspergillus nidulans orlA gene encodes the trehalose-6-phosphate phosphatase which catalyzes the last step of trehalose synthesis. We have isolated the *orlA* gene from a genomic cosmid library, and the nucleotide sequence of a 4.75-kb stretch of DNA covering the whole gene was determined. The *orlA* gene was predicted to contain a presumptive open reading frame of 2,646 bp which is interrupted by a 58-bp intron and encodes a protein of 882 amino acids. Under normal growth conditions, only low level of *orlA* expression was observed both in vegetative mycelia and conidiophores. However, the expression of *orlA*was increased by any of the environmental stresses, heat shock, oxidative stress, and osmostress. Interestingly, the increase of *orlA* expression was much more significant in the asexual organs. The conidia of *orlA* deletion mutant showed decreased levels of resistance against both heat shock and oxidative stress comparing with those of wild type. The sensitivity of the mutant to heat shock and oxidative stress was remedied by an osmotic stabilizer such as 0.6 M KCl. These results suggest that *orlA* mainly contributes to promote the resistance of the conidia against environmental challenges, such as heat shock, oxidative stress, and osmostress.

231. Inducible expression of *Aspergillus nidulans uvsJ* by mutagenic DNA-damage and specific accumulation of UvsJ protein in conidial nuclei. Su Jin Cho[1], Suhn Kee Chae[2] and Pil Jae Maeng[1]. [1]Department of Microbiology, Chungnam National University, Daejeon 305-764; [2]Division of Life Science, Paichai University, Daejeon 302-735, Korea.

Aspergillus nidulans uvsJ gene is known to encode a ubiquitin-conjugation enzyme and to be included in UvsF group, one of the four epistatic group of DNA repair genes. The presumptive functional analogue of *uvsJ, RAD6*, has been reported to be involved in various cellular processes, such as sporulation, DNA repair, ubiquitination, cell cycle control, in *Saccharomyces cerevisiae*. In the present study, we analyzed the expression of*uvsJ* and the localization of UvsJ protein using dimeric sGFP as a vital reporter. Under normal conditions, only a little transcription of *uvsJ* was detected through the whole thallus when monitored in both *uvsJ-p::(sgfp)*$_2$ and*uvsJ::(sgfp)*$_2$ transformants. On the other hand, when the cells of the transformants were subject to mutagenic DNA-damage rendered by alkylating chemicals (MMS, EMS, and 4-NQO), UV, and heat shock, the level of *uvsJ* expression was increased both in vegetative and asexually differentiated cells. Quite surprisingly, UvsJ::(sGFP)$_2$ protein was specifically accumulated in the nuclei of conidiospores under the conditions of mutagenic DNA-damage. These results suggest that the expression of *uvsJ* is controlled at both transcriptional and post-transcriptional levels.

232. Differential expression of four different chitin synthase genes of *Aspergillus nidulans* in response to developmental status and environmental stresses. Jeong Im Lee, Yoo Mi Rho, Min Young Lee, Beom Chan Park, Hee Moon Park, and Pil Jae Maeng. Department of Microbiology, Chungnam National University, Daejeon 305-764, Korea.

Aspergillus nidulans has five *chs* genes encoding chitin synthases. We analyzed the expression mode of the four of the five chitin synthase genes, *chsA*,*chsB*, *chsC*, and *chsD*, using a mutant version of green fluorescent protein (sGFP) as a vital reporter. *chsA* was expressed at a considerable level in whole conidiophores during asexual development, but was expressed little in vegetative hyphae and foot cells throughout the culture period. During sexual development, *chsA* was not expressed either in the mycelia induced to sexual development or in the sexual structures. Expression of *chsB* was not only ubiquitous throughout the fungal body including substrate mycelia and conidiophores but also regardless of the developmental status of the cells. *chsC* was expressed only at basal levels during vegetative growth and asexual development. However, during sexual development, *chsC* was expressed at a considerable level in the shells of young cleistothecia and was strongly expressed specifically in mature ascospores. *chsD* was expressed at basal level in conidiophores and vegetative hyphae during asexual development, and strongly expressed in the shells of young cleisthothecia and Hulle cells at the early sexual stage. The expression of the chitin synthase genes, *chsA*, *chsC*, and *chsD*, except for *chsB*, was altogether enhanced by high concentrations of salts, such as KCl and NaCl.

233. The PEST-2 element in FREQUENCY is required for expression of the *Neurospora crassa* photo receptor WHITE COLLAR-1. Tobias Schafmeier, Christian Mohr and Michael Brunner. Biochemie - Zentrum Heidelberg, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany

FREQUENCY (FRQ), White Collar 1 (WC-1) and White Collar 2 (WC-2) are central components of the circadian clock in Neurospora crassa. WC-1 and WC-2 form a complex (WCC) that drives expression of frq RNA. In the course of a circadian day FRQ is progressively phosphorylated and degraded. Two PEST elements in FRQ, PEST-1 and PEST-2, are phosphorylated in vitro by recombinant casein kinase CK-1a. Single or double deletions of the PEST sequences result in hypophosphorylation of FRQ protein in vivo and arrhythmic condiation of the mutant strains. We show that the two PEST elements have specific and distinct functions in the circadian feedback loop. As shown previously, deletion of PEST-1 leads to a reduced rate of FRQ protein turnover. The corresponding mutant strain accumulates high levels of FRQdeltaPEST-1 protein. Levels of WC-1 are also elevated in frqdeltaPEST-1 cells, supporting that FRQ is required for efficient WC-1 expression. Deletion of PEST-2 does not affect FRQ protein stability. frqdeltaPEST-2 cells express low levels of the mutant FRQ and significantly reduced levels of WC-1. Double deletion of PEST 1 and PEST-2 stabilizes the mutant protein and restores expression of high levels of FRQdeltaPEST-2 protein while WC-1 levels remain low. The data suggest that the PEST-2 region in FRQ is required for expression of WC-1 while PEST-1 regulates FRQ protein turnover.

234. Putative chromosome remodeling factors in *Neurospora crassa*: creation of ISWI-type ATPase mutants by RIP. Michael Freitag and Eric U. Selker, University of Oregon, Eugene.

Chromatin remodeling factors (CRF) utilize energy from ATP hydrolysis to induce directed nucleosome sliding and serve as global activators or repressors of gene expression. Four major classes of CRFs (SWI2/SNF2, ISWI, Mi-2/CHD, INO80) have been distinguished, based on the structure of their highly conserved ATPase subunits. We identified 24 predicted proteins with conserved SWI/SNF ATPase domains in the Neurospora genome. Unlike mammalian cells and *Saccharomyces cerevisiae*, Neurospora has few paralogous pairs of SWI/SNF ATPases, making Neurospora an attractive model system for genetic analyses of CRFs. In addition to single homologs of *S. cerevisiae SWR1* (Neurospora *crf1-1*), *INO80*(*crf2-1*), *SWI2/SNF2* (*crf3-1*), *ISW1* (*crf4-1*) and*CHD1* (*crf7-1*), Neurospora also has homologs of the animal Mi-2 (*crf6-1*) and Arabidopsis DDM1 (*crf5-1*) genes. Both Mi-2- and DDM1-type proteins have been implicated in the control of eukaryotic DNA methylation. To study involvement of the ISWI-type ATPases in DNA methylation in Neurospora we mutated *crf4-1* and *crf5-1* by RIP. A GFP-tagged *crf4-1* gene driven by the Neurospora *ccg-1* promoter was inserted at the *his-3* locus. The CRF4-1/GFP fusion protein was properly localized to the nucleus. Neurospora *crf4-1* is not an essential gene, which resembles findings with deletion strains of *S. cerevisiae ISW1* and*ISW2*, but is in contrast to Drosophila ISWI mutants. (Supported by NIH grant # GM35690 to E.U.S.)

235. Cloning of the SIP3 homologue in *Aspergillus nidulans*. [1]Shobana Krishnan*, [2]Rosanna Penna-Muralla and [3]Rolf Prade [1,2,3] - 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 subcloned 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. The *sipA3*gene comprises 5 exons interrupted by 4 introns. The predicted protein has high similarities with other filamentous fungi like *A.fumigatus* and *N.crassa*. A disruption cassette has been constructed with the selectable marker *argB*. A *sip3* disruption mutant has been created by transformation-mediated gene disruption using this cassette and its growth in various carbon-sources like glucose, sucrose and pectin has been studied and compared to wild type.

236. VIVID is a flavoprotein and serves as a fungal blue light photoreceptor for photoadaptation. Carsten Schwerdtfeger[1,2], Jennifer J. Loros[2], Jay C. Dunlap[2], and Hartmut Linden[1].[1]Lehrstuhl für Physiologie und Biochemie der Pflanzen University of Konstanz, Germany. [2] Department of Biochemistry and Department of Genetics, Dartmouth Medical School. Hanover NH. USA 03755.

Blue light regulates many physiological and developmental processes in fungi. Most of the blue light responses in the ascomycete *Neurospora crassa* are dependent on the two blue light regulatory proteins White collar-1 and 2. White collar-1 was recently shown to be the first fungal blue light photoreceptor. In the present study, we characterize the *Neurospora* protein VIVID which is also involved in the transduction of the blue light signal. VIVID reveals a partial sequence similarity with plant blue light photoreceptors. In addition, we found that VVD noncovalently binds a flavin chromophore. Upon illumination with blue light, VVD undergoes a photocycle indicative of the formation of a flavin-cysteinyl adduct. The VVD photocycle closely matches the photocycle of the plant blue light photoreceptor phototropin. VVD is localized in the cytoplasm and is only present after a light induction. A loss of function vvd mutant was completely insensitive to increases in light intensities. Our results suggested that VVD is a second *Neurospora* blue light photoreceptor, which enables *Neurospora* to perceive and respond to daily changes in light intensity.

237. Blue light regulation of DNA photolyase gene expression. Benjamin A. Horwitz, Gloria M. Berrocal-Tito, Inbal Marom, and Alfredo Herrera-Estrella[1]. Department of Biology, Technion - Israel Institute of Technology, Haifa 32000, Israel. [1]Department of Plant Genetic Engineering, Centro de Investigacion y Estudios Avanzados del I. P.N., Unidad Irapuato, Irapuato, Gto. 36500, Mexico.

The photolyases, DNA repair enzymes that use visible and long-wavelength UV light to repair dimers created by short-wavelength UV, belong to the larger photolyase-cryptochrome gene family. Blue light rapidly induces expression of a photolyase gene, *phr1*, of*Trichoderma atroviride* (1). The sensitivity to light is increased in transgenic lines overexpressing Phr1. This suggests that *phr1* encodes a unique protein, capable of light dependent autoregulation. If this model is correct, this would be the first member of the photolyase photoreceptor family with both DNA repair and sensory functions. Phr1 could provide a "memory" of recent exposure to light, through this sensory role. To further investigate the mechanism of photoregulation of the photolyase gene, we are introducing reporter constructs into *Trichoderma atroviride*, and comparing the light regulation of *phr1* with that of its orthologs in other species.

(1) Berrocal et al. J Biol Chem 274(1999) 14288; Photochem Photobiol 71(2000) 662

238. Functional analysis of the transcription factor *MST12* in *Magnaporthe grisea.* G. Park & J.R. Xu. Dept.Bot.& Plant Path., Purdue Univ., W. Lafayette, IN 47907

In *M. grisea*, *MST12* is a transcription factor that is essential for appressorial penetration and plant infection. In this study, we further characterized the function of *MST12* and the defects of *mst12* mutants. TEM examination showed

that*mst12* mutants formed appressorial pores but failed to develop penetration pegs. Preliminary data indicated that Mst12-GFP was weakly expressed in mature appressoria and became localized to the nucleus in the penetration peg. Structurally, *MST12* have a HD and zn-finger domains, and a middle region between them. Their effect on *MST12* function will be studied using mutants deleted of each domain. In the middle region, there is a MAPK, a PKA, and a PKC phosphorylation sites that are well conserved among filamentous fungi. We have generated *mst12* mutants with these phosphorylation sites changed individually by site-directed mutagenesis. Phenotypes of these mutants will be presented. In addition, when *MST12* was over-expressed in DH51, appressorial penetration and lesions were observed, indicating involvement of *MST12* in the cAMP signaling. Although *MST12* can weakly interact with *PMK1* in yeast two-hybrid assays, preliminary data indicate that overexpressing *MST12* can not complement the defect of *pmk1* mutant nn78. It is likely that there may be additional transcription factors involved in *PMK1* signaling for appressorium formation. One of the candidates is the *TEC1* homolog. Appressorium formation was significantly reduced in the *MgTec1* knock-out mutants.

Genomics and Proteomics

239. The *Ashbya gossypii* genome: lessons learned by comparison to the *Saccharomyces cerevisiae* genome. S. Brachat[1], F. S. Dietrich[1,3], S. Voegeli1, A. Lerch[1], T. Gaffney[2] and P. Philippsen[1]. [1]Biozentrum der Universität Basel, Basel, CH. [2]Syngenta, Research Triangle Park, NC, USA. [3]Current Address: Department of Molecular Genetics and Microbiology, Duke University Medical Centre, Durham, NC, USA.

We completed the sequencing of the 9Mb genome the filamentous fungus *Ashbya gossypii* which encodes 4720 protein coding genes, 190 tRNA genes, 50 small RNA genes and 40-50 copies of the rRNA genes. With respect to the size and the number of genes, this represents presently the smallest genome of a free living eukaryot. Surprisingly, the protein coding genes revealed striking similarities to that of *S. cerevisiae* with over 95% of the *A. gossypii* genes sharing significant homology to *S. cerevisiae* genes. In addition, 90% of *A. gossypii* genes show both, homology and synteny, with the genome of the baker's yeast. The synteny can be described as "relaxed synteny" since the gene order in any *A. gossypii* chromosomal region reflects the sum of the gene order of 2 different regions in the *S. cerevisiae* genome. This observation can only be explained by a whole genome duplication in the *S. cerevisiae* lineage followed by frequent deletion of one of the two gene copies. Thus, the *A. gossypii* genome becomes the strongest evidence for the yeast genome doubling hypothesis. Careful alignment of both genomes lead to the identification of most translocation and inversion events in the evolutionary history of both organisms (500 rearrangements) and of the complete set of still duplicated genes in *S. cerevisiae*. It also made possible the detection of potential annotation or sequencing errors in the yeast genome. 5% of the A. gossypii genes were not found in *S. cerevisiae* (NOHBYs NO Homologues in Baker's Yeast). Nearly 60% of the NOHBYs have homologues in other species. Functional assessment based on homology and domain searches demonstrated the presence of many transcription factors among NOHBYs as well as the presence of new gene families. Similarly, over 400 confirmed *S. cerevisiae* genes could not be found in the *A. gossypii* genome. Analysis of this difference in gene pool is likely to corroborate the difference in life style between the two organisms.

240. Analysis of Thiol Peroxidases in *Cryptococcus neoformans*. Tricia Missall, Mary Ellen Pusateri, and Jennifer Lodge. Biochemistry, Saint Louis University School of Medicine, St. Louis, MO.

Cryptococcus neoformans is a human fungal pathogen found worldwide, most commonly causing meningitis in immunocompromised patients. There are many factors that affect the virulence of *C. neoformans* including its ability to grow at 37℃. Two-dimensional gel electrophoretic analysis illustrates a difference in the protein expression of *C. neoformans*grown at 37℃ versus 25℃. MALDI-TOF analysis and sequence homology to other species, including *Saccharomyces cerevisiae,* identify these proteins as peroxidases that belong to the thiol specific antioxidant (TSA) family. Both of these enzymes, Tsa1 and Tsa3, are shown to be induced when *C. neoformans* is exposed to the oxidative stress of hydrogen peroxide. To determine the function of Tsa1 and Tsa3 in cryptococcal pathogenesis, deletion mutants of each and both of these enzymes were generated through homologous recombination at the*TSA1* and *TSA3* loci. The contributions of these thiol peroxidases to the virulence of *C. neoformans* were then analyzed by comparing the *in vitro* and*in vivo* growth of these mutants to that of the wild type strain. The *tsa1* mutant and *tsa1,tsa3* double mutant show sensitivity to both hydrogen peroxide and t-butyl hydroperoxide as well as significant growth retardation at high temperatures. In mice, the *tsa1* and *tsa1, tsa3*

mutants showed significantly less growth in the brain and lung (p tsa3 mutant grew only moderately less in the brain ($p < 0.05$) than the wild type strain. This is the first example showing the importance of a thiol peroxidase to the virulence of an organism.

241. Characterisation of appressoria specific proteins from *Phytophthora infestans*. Laura J. Grenville[1], Catherine R. Taylor[1], Alison Williams[1], Paul Birch[2], and Pieter van West[1].[1] University of Aberdeen, Department of Molecular and Cell Biology, Foresterhill, Aberdeen, AB25 2ZD. [2] Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland UK.

A thorough understanding of the molecular events taking place during early interactions between *P. infestans* and host and non-host plants is crucial for developing new control measures. The appressorial stage of the interaction is the first point in which direct contact between the pathogen and the plant occurs via the formation of highly specialised infection structures, such as the appressorium, penetration peg, and the infection vesicle. Moreover, it is during this phase that plant defence responses are initiated. Resistance is based, principally, on recognition of a particular elicitor component from the pathogen. Therefore, we anticipate that secreted and cell wall proteins from the appressorial infection stage of *P. infestans* are likely to be rich in important signalling molecules involved in disease resistance or establishing a successful infection process. A proteomic approach is employed to accelerate the discovery of novel extra-cellular and appressorial stage-specific proteins. Here we present our latest results.

242. A proteomic approach to identify secreted proteins from *Phytophthora infestans* relevant to the host-pathogen interaction. Shuang Li[1], Pieter van West[1], Sophien Kamoun[2], Neil A.R. Gow[1]. [1] University of Aberdeen, Department of Molecular and Cell Biology, Foresterhill, Aberdeen, AB25 2ZD, Scotland UK. [2] The Ohio State University, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691, USA.

Microbial pathogens of plants secrete avirulence factors that are recognised by the products of plant resistance genes and trigger host resistance responses. In order to identify potential avirulence factors from *Phytophthora infestans*, secreted protein profiles of several strains with different avirulence phenotypes were analysed by proteomics. About 500 proteins were identified from culture filtrates of each strain. The most abundant 50 protein spots from one strain were analysed by peptide fingerprinting using MALDI-TOF mass spectrometry. Comparative analysis of the profiles of strains with known avirulence characteristics identified several proteins that were expressed differentially. The possible function of the corresponding genes could not be determined from the DNA sequences. Northern analysis showed that some of the genes were differentially expressed in the tested strains under the same or different culture conditions. Functional analysis of these genes will establish their potential as avirulence determinants of *P. infestans*.

243. Differential Gene Expression in Germinating Basidiospores. Lori G. Baker[1], Sarah F. Covert[2], and Paula Spaine[3]. [1]Department of Genetics, [2]Warnell School of Forest Resources, [3]Forestry Sciences Laboratory, Southeastern Forest Experiment Station, USDA Forest Services. The University of Georgia, Athens, GA, U.S.A

Cronartium quercuum f.sp. fusiforme is the causative agent of the southern pine disease known as fusiform rust. Depending upon the environment, *C. q. fusiforme* basidiospores can germinate either directly or indirectly. When *C .q. fusiforme* basidiospores are cast upon pine needles or polystyrene they germinate directly by sending out a thin long germ tube. In contrast on non-host surfaces 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. The first part of this study is a comparison of gene expression patterns between the two germination fates. Suppression subtraction hybridization was used to make two cDNA libraries and a total of 1920 clones from them were sequenced. Sixty-eight of the clones are unique to the direct germination library, 82 are unique to the indirect germination library, and 15 are common to both libraries. Array hybridizations will be used to identify genes that are differentially expressed between directly and indirectly germinating basidiospores, germinating aeciospores, and pycniospore and aeciospore formation. 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 process as well as contribute to our general understanding of the same events in other rust/pathogenic fungi. The second part of this project tests the hypothesis that substrate hydrophobicity/wettability regulates basidiospore germination fate. The substrate surface wettabilities were altered as follows: polystyrene was exposed to ultra violet light to make it more hydraphilic and

glass was treated with silane to make it more hydraphobic. Basidiospore germination experiments on the altered surfaces are in progress.

244. The asexual pathogen *Aspergillus fumigatus* has the coding capacity for mating-type proteins, pheromones and pheromone receptors. Stefanie Poeggeler and Ulrich Kuck. Botany, Ruhr-University Bochum, Bochum, Germany.

The filamentous fungus *Aspergillus fumigatus* is one of the causes of invasive lung disease in immunocompromised individuals. It has been classified as asexual because no direct observation of mating or meiosis has been reported. Sequencing of the complete genome by an international collaboration including the Wellcome Trust Sanger Institute (UK) and The Institute for Genomic Research (TIGR, USA) has made most of the genomic sequence information from*A. fumigatus* publicly available. By searching the incomplete genome sequence of *A. fumigatus*, I have identified the coding capacity for a set of proteins that could be involved in mating and the pheromone response pathway. These include one putative mating-type gene, one gene encoding a pheromone and two pheromone receptor genes. The mating-type gene encodes a HMG domain protein exhibiting significant similarity with mating-type proteins from sexually reproducing filamentous ascomycetes. The pheromone gene is predicted to encode a precursor pheromone that is processed by a KEX2-like protease to yield a pheromone that is structurally similar to the alpha-factor of the yeast *Saccharomyces cerevisiae*. In addition, the deduced gene products of the receptor genes are putative seven-transmembrane proteins, which displays a high-level amino acid identity with the a-factor receptor Ste3p and the alpha-receptor Ste2p of *S. cerevisiae*, respectively. The identification of these homologues suggests the existence of a sexual cycle in *A. fumigatus*.

245. Identification of Perennial Ryegrass and Endophyte Proteins Involved in Symbiosis. Shalome Campbell[1,2], Mike Christensen[1], Richard Johnson[1], T. William Jordan[2] and Gregory Bryan[1]. [1]AgResearch Limited, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand; [2]Victoria University of Wellington, PO Box 600, Wellington, New Zealand

Perennial ryegrass (*Lolium perenne*) is one of New Zealand's most important pasture species. Most New Zealand ryegrass pastures are infected with *Neotyphodium lolii*, an endophytic fungus which grows intercellularly within the host thereby obtaining nutrients. In return the fungus produces a range of secondary metabolites which confer host resistance to a number of insect pests. We are developing 2D electrophoresis and proteomic techniques to identify components of this symbiotic relationship. Initial approaches focused on comparison of isogenic ryegrass lines with and without endophyte infection. We are targeting enriched protein fractions from plant tissues, including intercellular fluid from the apoplastic space of leaf and stem tissue. Using a combination of 2D electrophoresis and polyclonal antibodies to *N. lolii*, we have obtained mass fingerprint analysis for nine protein spots. Four of the nine spots were matched to plant proteins that are either up or down regulated during pathogenesis in other plant-microbe interactions. A further three protein spots have been identified by mass spectrometry after narrowing the pH range in the first dimension. We are currently using degenerate PCR to clone these gene products, two of which may be of fungal origin. Future work will focus on gene silencing to elucidate the role of these genes in symbiosis.

246. Expression Analysis of *Aspergillus oryzae* in Solid-state Fermentations. Rob te Biesebeke, Peter Punt, Ana Levin, Margreet van Heerikhuisen, Arjen Rinzema, Kees van den Hondel. Wageningen Centre for Food Sciences, The Netherlands, TNO Nutrition and Food Research Institute, The Netherlands, Food and Bioprocess engineering group, Wageningen University, The Netherlands

Fungal solid-state fermentations (SSF) are applied in many traditional food fermentation processes. There is abundant proof in literature that the product spectrum from SSF is very different from that obtained in submerged fermentation (SmF). However, the mechanisms underlying these differences are not at all understood. Therefore rational new design of SSF processes to make new products and optimise the production of existing products is not possible. Only recently, significant advances have been made in understanding the physical (process engineering) aspects of SSF but the information on physiology and molecular genetics is limited. We have initiated a comparative analysis of gene expression of the model organism Aspergillus oryzae in SSF and SmF. With the development of

fungal genomics technology a genome-wide approach can be followed to study SSF specific gene expression. Our results show a large difference in the spectrum of expressed genes in SSF and SmF as confirmed by Northern analysis. Our results indicate the presence of gene regulatory mechanisms specific for surface grown *Aspergillus oryzae* not observed in liquid cultures.

247. Genomic analysis, deletion and the characterization of Multidrug tranporters of a fungal plant pathogen, *Cochliobolus heterostrophus.* Uvini Gunawardena[1], Olen Yoder[1] and Gillian Turgeon[2] [1] Torrey Mesa Research Institute, San Diego, CA[2] Cornell University, Ithaca, NY

Eukaryotic membrane transporters include two major families of proteins that are involved in multidrug transport – ABC (**A** TP **B**inding **C**assette) and MFS (Major Facilitator Superfamily) transporters. In the context of plant pathogen interactions, these multidrug resistance proteins 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 toxins. We have mined the genomic sequence of*Cochliobolus heterostrophus*, a pathogen of corn, and found 41 putative ABC and 51 putative MFS proteins. All the ABC transporters of *C. heterostrophus* have been targeted for deletion and null mutants of 38 genes have been collected. Deletions of two ABC transporters are believed to cause lethality because of repetitive failures to obtain gene deletants. Interestingly, one of these transporters resides close to NPS9 (**N**on Ribosomal **P**eptide**S**ynthetase encoding gene) suggesting a role in the transport of a small peptide with cytotoxic properties, whose synthesis is controlled by NPS9. Deletion of two other ABC transporters resulted in reduced virulence of *C. heterostrophus* on corn. One is presumed to be involved in the efflux of phytoalexins from fungal cells. The other is hypothesized to have a role in the transport of NPS6, a virulence factor, thus mediating disease establishment. Characterization of these transporters including their role in fungal mating and resistance to cytotoxic compounds will be presented.

248. Conservation of Active Site and Essential Structural Components Encoded by a Putative *Neurospora crassa* Phytase Gene. Edward J. Mullaney and Catherine B. Daly. SRRC ARS USDA New Orleans, LA 70124

The *Neurospora crassa* sequencing project has identified many hypothetical proteins. One such protein, Locus NCU06351.1, has been reported as having the conserved sequence unique to the histidine acid phosphatase class of enzyme. *Aspergillus niger* phytase is a member of this class of enzyme. The gene for this enzyme has been cloned, over-expressed, and its product is now commercialized as an animal feed additive with an annual market value of over $$500 million. A study of the amino acid sequence of NCU06351.1 reveals additional evidence that it is analogous to the Aspergillus phytase. All the essential components of the active site and the substrate specificity site are present in this putative Neurospora protein. Remarkably, it also appears that all the constituents of the five disulfide bridges found in Aspergillus phytase have been conserved in this NCU06351.1. These disulfide bridges seem to have a vital role not only in the folding pathway, but also in maintaining the proper configuration of the molecule for catalytic activity. As more is learned about the structure and function of other fungal phytases, ascribing characteristics to this putative Neurospora phytase will become more precise.

249. Gene Ontology Tools at SGD. Chandra Theesfeld, Shuai Weng, Dianna Fisk, Rama Balakrishnan, Karen Christie, Kara Dolinski, Selina Dwight, Eurie Hong, Laurie Issel-Tarver, Anand Sethuraman, Gavin Sherlock, David Botstein and Michael Cherry. Department of Genetics, Stanford University

Every *Saccharomyces cerevisiae* protein is now associated with a biological process term(s) from the Gene Ontology (GO). In order to help biologists understand and utilize the ontologies created by the GO Consortium, the *Saccharomyces* Genome Database (SGD) has created two new web-based tools and a graphic display: the GO Term Mapper, the GO Term Finder and the GO Tree View. The GO Term Mapper and GO Term Finder tools were designed as aids to researchers employing large-scale methods of analysis. The GO Term Mapper allows users to input a list of genes and determine the upper level GO terms associated with those genes by tracing the ontologies from the granular, specific term (associated directly with a gene) to an upper level GO term. The upper level GO terms used by the GO Term Mapper are pre-defined by SGD and represent a low-granularity slice of the ontology (GO Slim). The GO Term Finder also allows users to input a list of genes, but rather than mapping the genes to a defined set of terms, this tool searches the GO structure to find significant GO terms shared among the genes in the list. The GO Tree View helps users place selected GO terms into context by graphically illustrating the terms within the GO structure; genes directly or indirectly associated with each term are also shown. To help familiarize users

with GO resources at SGD we have created a GO tutorial. The GO tutorial is available here: http://genome-www.stanford.edu/Saccharomyces/help/gotutorial.html The GO tools are available at the following URLs: http://genome-www4.stanford.edu/cgi-bin/SGD/GO/goTermMapper

http://genome-www4.stanford.edu/cgi-bin/SGD/GO/goTermFinder

The SGD home page is: http://genome-www.stanford.edu/Saccharomyces/

250. Prediction of Questionable Open Reading Frames. Hong, E.L., Dolinski, K., Balakrishnan, R., Christie, K. R., Costanzo, M. C., Dwight, S. S., Engel, S. R., Fisk, D. G., Sethuraman, A., Theesfeld, C. L., Weng, S., Botstein, D., and Cherry, J. M. Dept. of Genetics, Stanford University School of Medicine, Stanford, CA 94305

Several years after the completion of the systematic sequencing of the yeast genome, researchers are still questioning the total number of biologically significant open reading frames (biological ORFs) in *S. cerevisiae.* Recently, Jason Lieb and Patrick O. Brown (Dept. of Biochemistry, SUMC) proposed a scoring method involving multiple criteria such as ORF length, codon probability, and microarray data to discriminate between biological ORFs and those occurring by chance (questionable ORFs). We have expanded this idea to include other criteria and propose here a method for a more complete evaluation of questionable ORFs within the yeast genome. Our method of discrimination between biological and questionable ORFs included the presence or absence of *S. cerevisiae* ORFs in the genomes of other related species such as *S. paradoxus, S. mikatae, S. bayanus, Kluyveromyces yarowii, Schizosaccharomyces pombe,* and *Candida albicans*. ORFs were also validated based on the Gene Ontology (GO) function annotations described in the Saccharomyces Genome Database (SGD). Published data from large-scale analysis of *S. cerevisiae* genome were also given due considerations. ORFs were ranked for each criteria and all failure candidates were flagged questionable. Our ranking procedure to validate ORFs as well as the conclusions of our data analysis will be presented. SGD is supported by NHGRI.

251. Proteome analysis of extracellular proteins from solid-state culture of *Aspergillus oryzae*. Ken Oda, Kazuhiro Iwashita*, Dararat Kakizono, Haruyuki Iefuji, and Osamu Akita National research institute of brewing, 3-7-1, Kagamiyama, Higashihiroshima, Hiroshima, 739-0046, Japan

A. oryzae is an important microorganism for food industries, such as beverages and seasonings, and enzyme industries. The high productivity of various enzymes is the most important character of this industrial mold. For this importance, about 50 of genes encoding extracellular enzymes have been cloned and characterized. However, by the observation of 2D-electrophoresis, it is known there are still many kinds of proteins remain uncharacterized. Recently, more than 15,000 clones of EST sequence were reported and genome DNA sequencing project have been carried out by the Japanese consortium including universities, companies and national research industries. Using this DNA sequence database and mass spectrometry, we performed the identification of secreted proteins. The identification of proteins by mass spectrometry is based on the protease hydrolysis and released peptide mass. However, some extracellular proteins are highly glycosylated and resistant to protease hydrolysis. To overcome this problem, we developed in-gel deglycosylation method for increasing the efficiency of protease hydrolysis and decreasing the heterogeneity of peptide by the modification of oligosaccharide. Numbers of the detected peptides and the coverage of amino acid sequences were increased in several proteins by this treatment. Using this method, 41 proteins from extract of solid-culture were identified, in which 35 proteins have not been identified before.

252. Gene hunting in *Mycosphaerella graminicola*. Gert Kema[1], Els Verstappen[1], Theo van der Lee[1], Odette Mendes[1], Hans Sandbrink[2], Rene Klein-Lankhorst[2], Lute-Harm Zwiers[3], Mike Csukai[4], Ken Baker[4] and Cees Waalwijk[1]. [1]Biointeractions and Plant Health and [2]Greenomics, Plant Research International B.V., [3]Laboratory of Phytopathology, Wageningen University vand Research Center, P.O. Box 16, 6700 AA Wageningen, The Netherlands [4]Syngenta, Jealott's Hill, Berkshire, England

Septoria tritici leaf blotch of wheat, caused by *Mycosphaerella graminicola*, is a wheat disease of global importance. Approximately € 400 million which equals 30% of the total fungicide input in Western Europe, is used to control this disease. Although the interaction between *M. graminicola* and its host is microscopically well described, knowledge of *M. graminicola* genes involved in basic cellular functions as well as pathogenesis and

stress responses is limited. We aimed to fill this gap by a large EST project, providing sequence information of *M. graminicola* to underpin future control strategies. We produced EST libraries from seven in vitro conditions, representing various morphological forms and stress conditions, as well as three different interaction libraries to obtain the widest possible range of genes. Fungal clones were selected from the interaction libraries using a proprietary capture technology. In total, we generated 30.137 high quality reads of which 3894 were derived from in planta libraries. Based on the BLAST homology, clones selected from these libraries are largely of fungal origin. EST reads were assembled into 10.976 unigenes of which a large number were unique to the interaction libraries. A large proportion of the clones appeared to be full length, as exemplified for the elongation factor 1. We identified several novel ABC and MFS transporters as well as a comprehensive set of homologs of the various signal transduction pathways. In the in planta libraries a range of cellulytic enzymes was found. Further analysis of these genes by targeted disruption, expression analysis and comparative genomics is currently undertaken. Together with the wide body of sequence data currently available from related fungi this will boost our knowledge on the infection process of this important pathogen.

253. Diverse LTR-retrotransposons of the cultivated basidiomycete *Agaricus bisporus*. Richard W. Kerrigan[1] and Anton Sonnenberg[2]. [1]Sylvan Research, Kittanning, PA, USA. [2] Applied Plant Research BV, Horst, the Netherlands.

Screening of cosmid and lambda libraries of *Agaricus bisporus* (Lange) Imbach have revealed a diverse assemblage of LTR-retrotransposons, often with affinities to *gypsy* or *copia* elements. Based on the deduced amino acid sequences of ORFs corresponding to *gag* and *pol* genes, more than 10 distinct LTR-retrotransposons, either fragmentary or intact, are present in the genome of this mushroom. These elements are often present in high copy numbers and appear to be dispersed throughout the genome. It is common to find multiple elements represented within relatively short segments of chromosomal DNA. Fragments of elements also commonly disrupt the organization of other elements, making reconstruction of full-length sequences challenging. RT-PCR indicates that some of these retrotransposon genes are transcribed under certain conditions. Although we have no direct evidence for active transposition, some retroelements are associated with occasional alterations of the genome, based on data from Southern hybridizations to genomic DNA.

254. Deletion of *Aspergillus nidulans aroC* by a Novel Blaster Module Combining ET Cloning and Marker Rescue. Kerstin Helmstaedt, Sven Krappmann, Verena Grosse, and Gerhard H. Braus Institute of Microbiology and Genetics, Department of Molecular Microbiology, Georg-August University Goettingen, Germany

Blaster cassettes are of significant value in functional genomics, as they represent tools to inactivate duplicated or homologous genes in an individual organism subsequently. We have constructed a novel blaster module for the potential use of repeated gene deletion in the filamentous fungus *Aspergillus nidulans*. Due to the employment of bacterial resistance marker cassettes as direct flanking repeats, the blaster cassette is suited for recombinogenic engineering by ET cloning in *E. coli*. Functionality of the blaster module was demonstrated by deleting the chorismate mutase-encoding gene *aroC* of *A. nidulans* followed by marker rescue based on mitotic recombination. The resulting *aroC Delta* strains are auxotroph for phenylalanine but not tyrosine and display limited capacities of fruit body formation and ascosporogenesis, dependent on the phenylalanine/tyrosine supply. The data support the strong impact of the amino acid status on cleistothecia development in *A. nidulans*.

255. The role of lipid metabolism in virulence of *Candida albicans.* Katarzyna Piekarska and Marlene van den Berg, Guy Hardy and Ben Distel. Department of Biochemistry, Academic Medical Center, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

One typical characteristic of the pathogenic yeast *C.albicans* is its ability to grow in two different cell shapes, namely a yeast-form or a hyphal-form. Conclusive evidence has been provided that the ability to switch between these two morphologies is essential for its pathogenesis (1). Once initiated, the hyphal program not only results in the switch to the hyphal morphology to allow penetration of host tissues, but it also facilitates adaptation to the new environmental conditions, such as the host's internal lipid environment (2). Expression analysis showed that some peroxisomal enzymes are upregulated under those conditions notably two glyoxylate cycle enzymes: isocitrate lyase and malate synthase. Using a PCR based disruption technique we have constructed deletion strains of PEX5 and PEX13 genes, both of which are essential for peroxisome formation. The phenotypic characterization of these

C.albicans mutants will be presented. The second approach we are following is the application of DNA microarrays to identify hyphal specific genes encoding proteins involved in lipid metabolism. As a pilot for the expression analysis of the whole genome of *C.albicans* a set of genes was selected and used to generate submacroarrays to monitor differential gene expression during the yeast-to-hyphal transition in wild type and mutant *C.albicans* strains. 1. Lo, H.J. et al., Cell 90: 939-949, 1997 2. Lorenz, M.C. and Fink, G.R., Nature 412: 83-86, 2001

256. An ordered collection of expressed sequences from *Cryphonectria parasitica*. Angus L. Dawe, Vanessa C. McMains, Maria Panglao, Shin Kasahara, Bao Chen and Donald L. Nuss. Center for Biosystems Research, University of Maryland Biotechnology Inst, College Park, MD.

Cryphonectria parasitica is the causative agent of the chestnut blight disease. Earlier studies have demonstrated that *C. parasitica* represents a tractable model plant-pathogenic system. The availability of naturally occurring dsRNA elements (hypoviruses) that are capable of reducing fungal virulence provides the opportunity to examine molecular aspects of fungal plant pathogenesis in the context of biological control. In order to establish a genomic base for future studies of *C. parasitica*, we have analyzed a collection of expressed sequences (ESTs). A mixed cDNA library was prepared from RNA isolated from wild-type (virus free) and virus containing strains. Plasmid DNA was recovered from individual transformants and sequenced from the 5-prime end of the insert. Sequence data was trimmed of vector and poor quality information before being used to create a BLAST-searchable database of the complete EST dataset. Contig analysis of the collected sequences revealed that they represented approximately 2200 individual open reading frames. An assessment of functional diversity present in this collection was achieved by comparing the new sequence information against local (downloaded) copies of the NCBI protein database using the BLAST software utilities. The output was analyzed according to the significance likelihood of the hits returned for each clone. Using those hits deemed to be of greatest significance, this information was then classified according to the Gene Ontology Consortium guidelines for both molecular function and biological process. This data represents the largest collection of sequence information currently available for *C. parasitica* and is now forming the basis of further studies using microarray analyses to determine global changes in transcription that occur in response to hypovirus infection.

257. Exploring the pathogenesis of *Cryptococcus neoformans* using serial analysis of gene expression. Steen, B.R., Zuyderduyn, S., Lesiuk, J., Toffaletti, J., Rusaw, S., Marra, M., Jones, S., Perfect, J. and Jim Kronstad. University of British Columbia, Vancouver BC. Canada

Cryptococcus neoformans is a basidiomycete fungus that has emerged as an important opportunistic human pathogen of immunocompromised patients. *C. neoformans*, if inhaled, can disseminate from the lung to the blood stream where it is capable of crossing the blood-brain barrier. This often leads to fatal meningoencephalitis. To begin to explore transcription during growth of the fungus in the brain, *C. neoformans* H99 cells were harvested from the cerebral spinal fluid of twelve New Zealand white rabbits following intrathecal injection with $1.3x10^9$ H99 cells. Cells were harvested on days five ($2.6x10^7$ cells), seven ($1.1x10^7$ cells) and nine ($2.0x10^6$ cells) post infection. mRNA was isolated from a pooled collection of $3.9x10^7$ cells and the micro serial analysis of gene expression protocol (microSAGE) was used to construct a SAGE library. SAGE tags were collected, the most highly expressed tags were analyzed and the corresponding cDNAs or genomic contigs were identified. BLASTx was used to find similarities in the non-redundant database at the National Centre for Biotechnology Information (NCBI) website for each sequence. The most abundant tags *in vivo* were compared with their corresponding abundance profiles *in vitro*. The *in vitro* SAGE libraries were constructed from cells that were grown in minimal media at 25 and 37 degrees. In addition, tags that matched known genes were compared with the expression of these same genes in a serotype D strain of *C. neoformans*. Several genes that were highly expressed in *vivo* appear to be regulated differently between strains. It is possible that this may contribute to the higher degree of virulence for the serotype A strain relative to the serotype D strain. Several genes, four of which are HSP60, HSP90, HSP12 and the novel gene designated HOT1, have been chosen for further studies. These genes are being characterized in both serotypes to determine the role that these genes may play in the pathogenesis of C. neoformans and how differential regulation between serotypes may account for the differences in pathogenesis.

258. Development of expression systems for use in *Aspergillus fumigatus*. Michael J Bromley, Caroline L Gordon, Catherine E Rycroft, Peter M Hey and Jayne L Brookman. F2G Ltd., Manchester Incubator Building, Manchester, England.

Aspergillus fumigatus is an opportunistic fungal pathogen of man causing a wide spectrum of diseases some of which are fatal. To facilitate the discovery of efficient anti-fungal drugs we have identified genes that are required for fungal growth in vitro. To verify the potential of putative targets we have developed a conditional gene expression system in *A. fumigatus*. An important consideration for development was the ability of the expression system to act in any host models of fungal infection. We have previously demonstrated the use of wax moth larvae *Galleria mellonella* as a model for gene expression studies during *A. fumigatus* infection. The development of expression systems that can be manipulated in this and a more standard mouse model host are discussed in this study. The expression of a selection of *A. fumigatus* genes has been assessed in vitro. Two genes were identified (*cbaA* and *cbhB*) which were strongly expressed in the presence of an inducer (CMC) but repressed in the presence of glucose. Expression of *cbaA* and *cbhB* was not detected during infection of *G. mellonella*. Data will be presented on the expression of these genes during infection in the presence of the inducer. The promoter regions of these genes have been used as the basis for construction of expression cassettes in *A. fumigatus*. Data will be presented on the expression of a reporter gene in the cassette.

259. Microarray profiling of Hypovirus-infected Chestnut Blight Fungus, *Cryphonectria parasitica.* Todd D. Allen, Angus L. Dawe, Donald L. Nuss. Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742.

Members of the RNA virus family *Hypoviridae* persistently alter phenotypic traits, modulate gene expression and attenuate virulence of their fungal host, the chestnut blight fungus*Cryphonectria parasitica*. Creation of an ordered *C. parasitica* EST library/database consisting of 4200 sequences and representing approximately 2200 genes has facilitated the creation of a spotted cDNA microarray chip to examine global transcriptional responses to hypovirus infection. Infection by the severe hypovirus isolate CHV1-EP713 resulted in a significant alteration in transcript accumulation for approximately 10% of the arrayed ESTs, confirming and extending the results obtained from previous differential mRNA display analysis. In contrast, transcripts corresponding to only 5% of the spotted ESTs were found to be significantly altered in accumulation following infection by the mild hypovirus CHV1-Euro7. Of these genes, less than half were also altered in expression as a result of infection by the severe hypovirus CHV1-EP713. Thus, the changes in cellular transcriptional profiles caused by mild and severe hypoviruses differ considerably in both magnitude and composition. These results are consistent with previous observations of differential modulation of cellular signaling pathways by the mild and severe hypovirus isolates based on single gene readouts. Moreover, these surprising differences in the transcriptional profiles suggest a basis for differences in phenotypic changes caused by the two hypoviruses. The unique capabilities provided by the hypovirus/*C. parasitica* system for functional characterization of differentially expressed genes identified by microarray analysis will be discussed.

260. Functional genomics in *Magnaporthe grisea*: a strategic evolution in fungicide discovery. Anne Lappartient[1], François Villalba[1], Philippe Perret[1], Anne Marie Dechampesme[1], Christophe Fargeix[1], Mathieu Gourgues[1], Andy Tag[2], Sophie Rozenfeld[1], Jean Luc Zundel[1], Alain Villier[1], Terry Thomas[2], Marc Henri Lebrun[1], Roland Beffa[1]. 1 CNRS-UMR 1932 and Bayer CropScience, 14 rue P. Baizet, 69009 Lyon, France. 2 Texas A&M University, College Station, TX 77843-3258, USA

Novel modes of action are needed to overcome resistance to existing fungicides. Combinations of classical biochemistry tests together with genomic analysis are required for their identification. Transcriptome, proteome and metabolite analysis of fungi treated with antifungal compounds with unknown mode of action revealed the pathway inhibited. The validation of these results by classical biochemistry (e.g. enzyme activity test) as well as by functional genomic led to the enzyme inhibited by the tested compounds. Unraveling functions implicated in the infection process of plant or fungal growth is an important challenge for crop protection. Availability of the fungal genome sequences, such as *Magnaporthe grisea*, will facilitate the discovery of such genes. Engineered fungal transposons such as impala, an autonomous TC1/mariner element from *Fusarium oxysporum*; or Agrobacterium mediated transformation are versatile tools for insertion mutagenesis and gene replacement. Alternative methods using RNAi induced gene silencing were developed to inactivate rapidly genes of interest. Mutants expression profiles highlight pathways controlled by genes required for pathogenicity or growth and can be compared with those obtained with known inhibitors.

261. Identifying protein interaction networks in signaling pathways that regulate appressorium development in the rice blast pathogen *Magnaporthe grisea*. Resham Kulkarni and Ralph Dean, Fungal Genomics Laboratory, North Carolina State University.

Magnaporthe grisea, the causative agent of the rice blast disease, produces a specialized infection structure the appressorium to penetrate plant tissues. The cAMP signal transduction pathway is one of the signaling pathways that controls appressorium development. To identify new components within this pathway, we used the yeast two-hybrid system to screen adenylate cyclase (MAC1) and the catalytic subunit of cAMP-dependent protein kinase A (CPKA), against an appressorial cDNA library. The appressorial cDNA library was constructed in the GATEWAY donor vector (Invitrogen), enabling its transfer to different vectors by recombinational cloning. The protein phosphatase domain in MAC1, unique to fungal adenylate cyclases, interacted with a MAP kinase kinase and a Ser/Thr protein kinase. These interactions could be a part of feedback between the different pathways. A putative extracellular membrane protein ACI-1, which may serve as a cell receptor or an adhesion molecule, was also isolated as a MAC1 interacting protein. This protein contained an extracellular cysteine-rich domain, which also appears in a number of other fungal membrane proteins including *M. grisea* Pth11. The glutamate-rich N-terminus of CPKA interacted with a putative transcriptional regulator and two different glycosyl hydrolases. Phosphorylation motifs in these sequences suggest that they could be CPKA substrates. We are now scaling up the protein interaction assays to delineate interactions both in and between signaling pathways that are implicated in fungal development.

262. SAGE analysis of iron-regulated transcription in the human pathogenic fungus *Cryptococcus neoformans*. T. Lian, M., Zuyderduyn, S., Marra, S. Jones and J. Kronstad. Biotechnology laboratory, Department of Microbiology and Immunology, and Faculty of Agricultural Science, The University of British Columbia, Vancouver, B.C V6T 1Z3, Canada. Genome Sciences Center, B.C Cancer Agency, Vancouver, B.C. V5Z 4E6, Canada

The basidiomycete fungus *Cryptococcus neoformans* is an opportunistic pathogen that causes meningioencephalitis in immunocompromised individuals. The extracellular polysaccharide capsule produced by *C. neoformans* is essential for virulence and capsule production can be induced by iron starvation. We used the technique of serial analysis of gene expression (SAGE) to analyze iron-regulated gene expression in *C. neoformans*. Specifically, we constructed and characterized SAGE libraries from *C. neoformans*serotype D cells grown in low iron and iron replete media. This work provides the first analysis of the most abundantly transcribed genes for two different iron levels in this fungus. In addition, sets of genes that are implicated in iron metabolism were identified, including those encoding an iron permease (Ftr), a multicopper oxidase (Fet3), a ferric reductase, and an aconitase. Northern blot analysis confirmed that transcript levels for these genes were increased or decreased in response to iron deprivation. From the analysis of the most highly expressed tags under iron deprivation, a gene for a novel iron regulatory protein (IRP) was identified and analyzed. The disruption of the IRP gene in the strains B3501 and ATCC24067 resulted in cell growth arrest and loss of the ability to form enlarged capsule in iron-limiting conditions. These results suggest that IRP acts to facilitate or regulate the uptake of iron from the environment, perhaps acting as a sensor of iron availability. Further functional analysis of this gene and virulence tests in animals are in progress. These studies will contribute to an understanding the iron regulation of capsule synthesis and identify potential targets for anti-fungal therapy.

263. Characterization of the genetic interval spanning the *Avr1a* locus from*Phytophthora sojae*. D. Qutob[1], T. MacGregor[1], Y. Cui[1], T. Sharifian[1], and M. Gijzen[1]. [1]Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON N5V 4T3, Canada.

Phytophthora sojae is an aggressive soil-borne pathogen that causes damping-off diseases of soybean. In this patho-system, host resistant (*Rps*) genes and pathogen avirulence (*Avr*) genes determine race-cultivar specificity. Here we report progress on a map-based cloning study to isolate avirulence gene, *Avr1a*. Previously, a high-resolution genetic map of the region was constructed using DNA markers. A marker co-segregating with*Avr1a* was used to screen a bacterial artificial chromosome (BAC) library. Subsequent steps to chromosome walk resulted in an assembly of eight overlapping BAC clones spanning 170 kb and encompassing the *Avr1a* locus. A total of 119 kb of this contig has been fully sequenced and examined for predicted open reading frames (ORFs) and matches to expressed sequence tags. Among the 25-30 ORFs identified, there are few that show any similarity to known proteins. Three of the ORFs match expressed sequence tags from zoospore or infected plant cDNA libraries. At least

four other ORFs appear to represent expressed genes, by RT-PCR analysis. A total of 10 of the potential ORFs share sequence similarities with one another, and constitute part of a large gene family that is clustered in the region. Currently, we are developing more markers along the contig and creating F_3 populations from recombinant F_2's to delineate the exact boundries of *Avr1a*. This information will facilitate identification of candidate genes to be screened for functional analysis and transformation.

264. Proteomics Of *Magnaporthe grisea*: Liquid Chromatography Mass Spectrometry For The Identification Of Extracellular Proteins. Sheng-Cheng Wu, Jeremi Johnson, Kumar Kolli, Peter Albersheim, Alan Darvill and Ron Orlando. Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602, USA

Extracellular proteins (ECPs) serve diverse and essential biological functions in various cell types at different physiological states. In fungi, ECPs are required for uptake of molecules for nutrition, growth, cell sensing and communication. Perhaps most importantly, many ECPs play crucial roles in fungal pathogenicity and fungus-host interactions. Despite their biological significance, very little is known about fungal ECPs. As fungal genomics enters a new era, it has become apparent that development of high-throughput proteomics technologies for systemic identification and profiling of fungal ECPs and other network proteins is required. We will report the use of 2-dimensional gel electrophoresis (2DE) to display, and liquid chromatography tandem mass spectrometry (LC MS/MS) to identify and archive *Magnaporthe grisea*ECPs from various culture conditions. *[This research was supported by U.S. Department of Energy (DOE) grant DE-FG05-93ER20221, the DOE-funded (DE-FG02-93ER20097) Center for Plant and Microbial Complex Carbohydrates, The National Science Foundation (NSF grant number 9626835), and the National Institutes of Health (NIH grant number P41RRR05351).]*

265. Gene expression studies in the soybean rust pathogen *Phakopsora pachyrhizi*. Martha L. Posada[1], Laura J. Ewing[2] and Reid D. Frederick[3]. [1]DOE Joint Genome Institute. 2800 Mitchell Drive, Walnut Creek, CA 94598. [2]Hood College, 401 Rosemont Ave, Frederick, MD.[3]21701USDA-ARS Foreign Disease-Weed Science Research Unit, 1301 Ditto Avenue, Fort Detrick, MD 21702.

Soybean rust is caused by the obligate fungal pathogen *Phakopsora pachyrhizi*. To understand gene expression during spore germination, a unidirectional cDNA library was constructed using mRNA isolated from *P. pachyrhizi* urediniospores germinating on a water surface. Sequence analysis of 908 clones revealed that 404 Expressed Sequence Tags (ESTs) displayed significant similarities to sequences in the "non-redundant" protein database. 56.5 percent of the identified ESTs were either hypothetical proteins or proteins of unknown function. The remaining 43.5 percent of the 404 ESTs were arranged using the Expressed Gene Anatomy Classification (EGAD) scheme. Approximately 20 percent of these genes were involved in metabolism, 7.5 percent in gene/protein expression, 5.5 percent in cell structure/motility, 4.5 percent in cell division, 3.5 percent in cell/organism defense, and 3.0 percent in cell signaling/cell communication. 488 unique ESTs were identified from the 908 clones, of which 380 ESTs appeared as a single copy. Several ESTs were identified as potential gene family members (i.e. gEgh16, DAHP synthase and non-histone chromosomal proteins). Expression of selected ESTs was measured during the infection process using real time RT-PCR.

266. Proteomic analysis of the *Neurospora crassa* cell wall. C. Alexander Valencia[1], John F. Kelly[2] and P. John Vierula[1]. [1]Department of Biology, Carleton University, Ottawa, ON, Canada, K1S5B6. [2]Institute for Biological Sciences, National Research Council, Ottawa, ON, Canada, K1A0R6.

The *Neurospora crassa* cell wall has a complex, laminate structure composed primarily of polymeric, mixed glucans and chitin, as well as a minor fraction of proteins which is poorly understood. A large number of proteins were extracted from purified cell walls by various chemical treatments, including urea and 2-mercaptoethanol, and separated by two-dimensional polyacrylamide gel electrophoresis. Altogether 89 proteins were examined by on-line liquid chromatography tandem mass spectrometry (LC-MS/MS) and amino acid sequences were obtained for 56 proteins. Fifty six proteins were identified by MASCOT searches against the NCBI and Whitehead Institute *Neurospora* protein databases, including known cell wall proteins, glycolytic enzymes, heat shock proteins and proteins involved in several other processes. Examples of identified proteins include enolase, glyceraldehyde-3-phosphate dehydrogenase, heat shock 70 kDa protein, elongation factor 1-alpha, outer mitochondrial membrane porin, alcohol dehydrogenase and NMT1. Moreover, this approach identified several novel proteins which could be involved in cell wall biogenesis.

267. EST analysis of the human pathogen *Paracoccidioides brasiliensis* yeast phase: identification of putative homologues of *Candida albicans* virulence/pathogenicity genes. Gustavo H. Goldman[1], Regina C. de Oliveira[3], Luiz R. Nunes[3], Luiz R. Travassos[4], Rosana Puccia[4], Fredj Tekaia[5], Marina P. Nobrega[6], Francisco G. Nobrega[6] and Maria Helena S. Goldman[2].[1]FCFRP and [2]FFCLRP, Universidade de São Paulo, Brazil;[3]Univ.Mogi Cruzes, Brazil; [4]UNIFESP, Brazil;[5]Institut Pasteur, France; [6]UNIVAP

Paracoccidioides brasiliensis is the causative agent of the prevalent systemic mycosis in Latin America, paracoccidioidomycosis (PCM). We present a survey of ESTs in the yeast pathogenic phase. We obtained 13,490 ESTs from both 5' and 3'-ends. Clustering analysis yielded the partial sequences of 4,692 genes. We have identified several *Candida albicans* virulence/pathogenicity homologues in *P. brasiliensis*. We have analyzed the expression of some of these genes during the transition yeast-mycelium-yeast (Y-M-Y) by real-time quantitative RT-PCR. Clustering analysis showed that mycelium-yeast transition revealed three groups: (i) RBT, hydrophobin, and isocitrate lyase; (ii) malate dehydrogenase, Contigs Pb1067 and Pb1145, GPI, and alternative oxidase; and (iii) ubiquitin, delta-9-desaturase,HSP70, -82, and -104. The first two groups displayed high mRNA expression in the mycelial phase, while the third group had higher mRNA expression in the yeast phase. Our results suggest the possible conservation of pathogenicity/virulence mechanisms among fungi.

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268. Sequencing the *Podospora anserina* genome. Philippe Silar[1], Christian Barreau[2],Véronique Berteaux-Lecellier[1], Michèle Chablat[1], Véronique Contamine[1], Evelyne Coppin[1], Corinne Clavé[2], Fons Debets[3], Robert Debuchy[1], Rolf Hoekstra[3], Marguerite Picard[1], Annie Sainsard-Chanet[4], Sven J. Saupe[2], Carole H. Sellem[4], Béatrice Segurens[5] and Jean Weissenbach[5].[1]Institut de Génétique et Microbiologie, UMR CNRS-Université 8621, Bâtiment 400, Université Paris-Sud, 91405 Orsay, France. [2]Institut de Biochimie et Génétique Cellulaire, UMR CNRS-Université 5095, Université de Bordeaux 2, 33077 Bordeaux, France. [3]Laboratory of Genetics, Wageningen University, arboretumlaan 4, 6703 BD Wageningen, The Netherlands.[4]Centre de Génétique Moléculaire, UPR CNRS 2167, 91198 Gif sur Yvette, France. [5]Génoscope, Centre National de Sequençage, 2 rue Gaston Crémieux, 91057 Evry, France.

P. anserina is used as a model in studies on prion propagation, cell degeneration, vegetative incompatibility, translation, sexual development and stability of mitochondrial DNA. We have started a 3-fold genome sequencing project. Our approach is a whole genome shotgun strategy combined with the sequencing of 5000 BAC extremities. Sequencing should be completed within one year. This work was supported by the Ministère de la Recherche et des Nouvelles Technologies and the Centre National de la Recherche Scientifique (ACI Séquençage à Grande Echelle) and by the Fondation pour la Recherche Médicale (Grant ñ EXC2001215009/1).

269. Transcript Profiling of Shiitake Mushroom *Lentinula edodes* revealed by Differential Display, cDNA Microarray and Serial Analysis of Gene Expression (SAGE). H. S. Kwan, W. Y. Chum, X. L. Bian, W. J. Xie, T. P. Ng, W. L. Ng, M. D. Zhang, G. S. W. Leung, S.M. Shih. Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong SAR, PRC

Development of mushrooms is an important area in mushroom biology being studied at the molecular level.We aim to characterize gene expression profiles during development of Shiitake mushroom *Lentinula edodes*.First, we used RNA fingerprinting with arbitrarily primed polymerase chain reaction (RAP) to isolate genes differentially expressed in fruiting. Over 100 genes were sequenced and about 15 genes were analysed. Second, RAP products were used as probes to hybridize cDNA macroarray membranes to identify differentially expressed clones. More than 100 genes were analysed. Third,500 unique Expressed Sequence Tags (ESTs) were generated from a primordium cDNA library. Differential expression of the ESTs were analyzed by cDNA macroarray and microarray analysis.To determine the proportions of each mRNA transcript among total transcripts, we used Serial Analysis of Gene Expression (SAGE).About 7,000 transcripts were counted from the mycelium and primordium and 110 genes couldbe identified.The genes abundantly and differentially expressed in primordium indicated that at the initiation of fruit body:(1)Specific sets of genes expressed in primordium,(2)mycelium-specific genes suppressed,(3)different sets of structural proteins appeared in different stages, (4) protein turn-over increased, (5) protein synthesis increased,and (6) signal tansduction increased. Differentilly expressed genes in the dikaryotic comparing to

monokaryotic mycelia has also been studied using a cDNA microarray printed with 2000 cDNA clones from a subtraction cDNA library.

270. Identification and Characterization of Differentially Expressed Genes in Dikaryons of *Lentinula edodes* by cDNA Microaray. S.M. Shih and H.S. Kwan Department of Biology, the Chinese University of Hong Kong, Shatin, N.T., Hong Kong Sar, PRC

Lentinula edodes (Shiitake mushroom) is a popular edible mushroom for its high nutrition and medical values. Fruiting process can only evolve from the dikaryons. Morphological and biochemical differences among dikaryons and monokaryons which relate to fruiting process can be analysed by generating the gene expression profiles of dikaryons. A subtraction cDNA library was constructed by multiple subtractions of a dikaryon, L54, with RNA from its two monokaryotic parents, A and B. Over 9000 cDNA clones were randomly picked and dotted on nylon membranes with a robot. The membranes were screened with probes of PCR products of total RNA of L54, A, and B to obtain a total of 1800 cDNA clones. These clones were printed onto glass chips to produce m microarrays. Fluorescent labeled probes were synthesized from total RNA of L54, A, and B, and hybridized to the cDNA microarray. Signal was amplified with tyramide and scanned and analysed. After normalization, among the 1800 dikaryotic clones, 20.3% was up-regulated and 16.6% down-regulated, and 23.6% was up-regulated and 13.2% down-regulated clones in L54 when compared with A and B respectively. The up-regulated clones were selected and sequenced. The genes sequenced were analysed with BlastX and matched with known structural proteins such as hydrophobin 2 and 3, extensin, and genes related to specific biochemical processes such as calcium binding protein, glutamate decarboxylase, heme-binding protein and pyruvate dehydrogenase.

271. Fungal proteomics: initial mapping of biological control strain *Trichoderma harzianum*. Jasmine Grinyer[1], Ben R. Herbert[1], Helena Nevalainen[2]. [1] Proteome Systems, North Ryde, Sydney, NSW, Australia. [2] Department of Biological Sciences, Macquarie University, Sydney, New South Wales, Australia

Trichoderma harzianum is a biological control agent applied against plant pathogenic fungi causing economical losses worldwide. We are using proteomics to identify key proteins involved in host recognition and biological control in a strain of *T. harzianum* with well-established biocontrol properties. Isolation of the corresponding genes will become a vital tool for developing genetically improved strains. One of the challenges with filamentous fungi is the solubilisation of proteins especially from the fungal cell envelope. We have found that an alkaline extraction pH does not favour the solubility of alkaline proteins, because they are almost uncharged at the extraction solution pH. Therefore, we have developed a method of treating a sample to extract proteins under acidic conditions. The method increases the solubilisation of alkaline proteins and eliminates acidic cell wall artifacts from microorganisms in general. Using this technique, combined with the use of protease inhibitors during sample preparation, thousands of proteins from *T. harzianum* have been extracted and separated using two-dimensional gel electrophoresis (2D PAGE). These proteins were identified using a combination of MALDI MS (matrix assisted laser desorption ionization mass spectrometry) and LC MS/MS (liquid chromatography mass spectrometry). Manual de novo sequencing was conducted to obtain sequence tags on unidentified proteins. Using these techniques, 24 protein spots were positively identified derived from 17 gene products.

272. *Fusarium* virulence and plant resistance mechanisms: a project within the Austrian genome programme GEN-AU. Gerhard Adam and Josef Glössl. Center of Applied Genetics, University of Agricultural Sciences, Vienna, Austria,

In 2002 the Austrian Federal Ministry for Education, Science and Culture has established the national genome programme GEN-AU (http://www.gen-au.at/). In a small pilot project researchers from the Center of Applied Genetics of the University of Agricultural Sciences, Vienna (CAG, coordinator), the Technical University Vienna (TU), the Institute for Agrobiotechnology in Tulln (IFA), the Austrian Research Center Seibersdorf (ARCS), and from the wheat breeding company Saatzucht Donau (SZD) will collaborate on several aspects. A prime goal is the development of efficient gene disruption methods for *F. graminearum* (CAG, TU). Mutants will be tested for altered virulence and altered metabolite production by LC-MS-MS (IFA). Also analytical techniques and reference materials for "masked mycotoxins" will be developed. *Arabidopsis thaliana* genes encoding mycotoxin inactivating enzymes will be characterized, and the role of zearalenone in plants will be investigated (CAG). The group from ARCS will work on the identification of differentially expressed genes in wheat and the development of DNA

arrays. The genetic basis of so far uncharacterized highly *F. graminearum* resistant wheat genetic resources will be determined (IFA), the knowledge gained about resistance QTLs will be utilized by the commercial partner (SZD). The aim of the GEN-AU program is "to secure and expand Austria's competitiveness and ability to cooperate on an international level". Collabrations are welcome!

273. Construction and validation of *Aspergillus nidulans* microarrays. Manda E. Gent[1], Andrew Sims[1], Geoffrey Robson[1], Geoffrey Turner[2], Rolf Prade[3], Hugh Russell[3], Nigel Dunn-Coleman[4], Stephen G. Oliver[1] . [1]School of Biological Sciences, Stopford Building, University of Manchester, Manchester, UK. [2]Department of Molecular Biology & Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, UK. [3]Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA. [4]Genencor International Inc, 925 Page Mill Road, Palo Alto, CA 94304, USA.

The use of microarrays in the analysis of gene expression is becoming widespread for many organisms including yeast. However, although a number of filamentous fungi have been fully or partially sequenced, microarray analysis is still in its infancy in these organisms. Here we describe the construction of microarrays for the fungus *Aspergillus nidulans*. A set of 4100 ESTs were isolated from conidial cDNA libraries and amplified by PCR. PCR products in 50% (v/v) DMSO were spotted onto Corning CMT-GAPS II slides using a Biorobotics Total Array System robot in 16 blocks of 17x16 spots 200mm apart. An experiment was designed to validate these arrays by monitoring the expression profile of known genes following the addition of 1% (w/v) glucose to wild-type A.nidulans cultures grown to mid-log phase in Vogel's minimal medium with ethanol as sole carbon source. Biomass samples for RNA extraction were taken from a 2 litre fermenter immediately before, (t=0) and at 1 hour, 2 hours and 4 hours after the addition of the glucose. RNA was extracted from biomass samples flash frozen in liquid nitrogen, labelled with cy3 or cy5 fluorescent dyes and hybridised to the arrays. Hybridisation profiles were analysed and quantified using Genepix software. The expression profiles following the glucose upshift will be presented and an assessment of the quality and reproducibility of the *A.nidulans* arrays discussed.

274. The *Paxillus involutus* / *Betula pendula* symbiosis: Gene expression in ectomycorrhizal root tissue. Tomas Johansson, Antoine Le Quéré, Derek Wright, Dag Ahrén, Bengt Söderström, and Anders Tunlid. Microbial Ecology, Lund University, Sweden

Ectomycorrhizas (ECM) are symbiotic associations formed between plants and soil fungi. In order to obtain information on genes specifically expressed during ECM symbiosis, transcript profiling in mycorrhizal root tissue was conducted. By Expressed Sequence Tag (EST) analyses 3555 clones were sequenced from a cDNA library constructed from ECM formed between the basidiomycete *Paxillus involutus* and birch (*Betula pendula*). In parallel, cDNA libraries from saprophytically growing fungus (3964 ESTs) and from axenic plants (2532 ESTs), respectively, were analysed. By clustering of all ESTs obtained (10,051), 2284 unique transcripts, either of fungal or plant origin, were discovered. From sequence homology analyses (GenBank (nr) protein database) a majority of these transcripts could be assigned putative functional and metabolic roles. This unique set of genes was further analyzed using cDNA microarrays on which a RNA target from the mycorrhizal root tissue was compared to RNA isolated either from the free-living fungus or the plant, respectively. From these analyses we found 170 fungal and 230 plant genes significantly up or down regulated (>2-fold) during mycorrhizal conditions. By comparison of EST and microarray data, we found a 78% agreement in results using the two different methods. Among regulated genes there was a significant portion being putatively involved in cell rescue and defence, metabolism and protein synthesis. Nevertheless, 59% of the fungal and 47% of the plant transcripts, respectively, were orphans without any recognized cellular function and could represent a rich source of information for future exploration on mycorrhizal symbiosis.

275. Identification and functional analysis of secreted proteins from *Magnaporthe grisea*. Cristina Filippi, Guodong Lu, Kiran Bhatterai, Elena Kolomiets and Daniel J. Ebbole. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA

Secreted proteins are candidates for eliciting defense responses or acting as virulence/aggressiveness factors in fungus/plant interactions. Virtually nothing is known about the range of secreted proteins that may function in such capacities. Using the available*Magnaporthe genome* sequence and an extensive collection of EST sequences we used available computer algorithms to make genome-wide predictions of secreted proteins in the genome.

Magnaporthe is predicted to have twice as many secreted proteins as its close relative, *Neurospora crassa* (~740 vs ~370). A comparison of *N. crassa* and *M. grisea* predicted secreted proteins reveals interesting differences (gain/loss, family expansion), but no clear means of distinguishing pathogenicity-related secreted protein factors from 'saprophytic' secreted proteins. As part of a major functional genomics project funded by NSF, we are cloning and expressing ~300 proteins in *Magnaporthe* to functionally test computer algorithm predictions for secretion of fungal proteins. The proteins are being directly tested for the ability to evoke a reaction on plants. We present a progress update as well as our current annotation of secreted proteins in *N. crassa* and *M. grisea*.

276. A Second Generation Genetic Map of *Gibberella zeae*. R.L. Bowden[1], J.E. Jurgenson[2], J.K. Lee[3], Y.-W. Lee[4], S-H Yun[4], K.A. Zeller[3], & J.F. Leslie[3]. [1]USDA-ARS Plant Science & Entomology Res. Unit, 4008 Throckmorton Hall, Manhattan, KS, [2]Dept. of Biology, Univ. of Northern Iowa, Cedar Falls, IA, [3] Dept. of Plant Pathol., Kansas State Univ., Manhattan, KS,[4]School of Agric. Biotechnol., Seoul National Univ., Su-won, Korea.

We have reported a genetic map of *G. zeae* made by crossing nitrate non-utilizing (*nit*) mutants of phylogenetic lineages 6 and 7, which contains 1048 AFLP markers assigned to nine linkage groups, but which contains regions with distorted segregation ratios and two possible chromosome rearrangements. We have now constructed a second generation genetic map by crossing two lineage 7 strains (Z-3639 & PH-1). The Z-3639 derivative strain used was heterothallic due to deletion of the *MAT2* gene. Thus, this cross avoids the segregation distortion accompanying selection for prototrophic *nit* progeny, and permits mapping of the *MAT* locus in *G. zeae*. AFLP marker polymorphism in this cross is lower, but no segregation distortion is occurring. At present the second map has 236 markers in 16 linkage groups, with more markers needed to saturate the map and consolidate linkage groups. Common AFLP loci can be used to synonimize linkage groups and for studies of the putative chromosome rearrangements and previous segregation distortion. The maps can be used for "forward genetics," e.g., QTL analysis of aggressiveness in natural variants.

277. Proteomics studies in *Trichoderma reesei*. Tiina Pakula, Markku Saloheimo and Merja Penttilä. VTT Biotechnology, P.O. Box 1500, 02044 VTT, Finland

Proteome analysis is the simultaneous examination of a large subset of the protein species in a given cell. Traditionally proteomics is performed by two-dimensional gel electrophoresis combined with protein identification by mass spectrometry. We have set up proteomics methodology for the analysis of both extracellular and intracellular proteins of *Trichoderma reesei*. We use 2-D gel electrophoresis at different pI ranges accompanied with staining with silver stain or the fluorescent dye Sybro ruby. Also protein samples from *in vivo* labelling with ^{35}S-methionine have been analysed to obtain more sensitivity and to study the synthesis rates of different proteins. Moreover, we have used antibodies against phosphoserine, phosphothreonine and phosphotyrosine to reveal changes in protein phosphorylation. The major cellulases produced by *T. reesei* can be readily recognised in 2-D gels run from both extracellular and intracellular samples. The cellulases, as most extracellular proteins are secreted from *T. reesei* as multiple pI forms. Deglycosylation of the proteins, e.g. the major cellulase CBHI, turns the protein into a single pI form, showing that the pI heterogeneity resides in the glycans. Our proteomics studies from intracellular samples have mostly been focused on analysis of secretion stress, i.e. the effects of compromised protein folding, processing or transport in cells producing heterologous protein or cells exposed to inhibitors of the secretory functions. These studies have pointed out a large number of protein spots affected by secretion stress. Major differences in the protein phosphorylation patterns also occur in these experimental conditions.

278. Identification of essential genes in the human fungal pathogen *Aspergillus fumigatus* by transposon mutagenesis. Arnaud Firon (1), François Villalba (2), Roland Beffa (2), and Christophe d'Enfert (1). (1) Institut Pasteur, Unité Postulante Biologie et Pathogénicité Fongiques, Paris, France, (2) Bayer CropScience, Biochemistry and Molecular Biology Department, Lyon, France

The opportunistic pathogen *Aspergillus fumigatus* is the cause of the most frequent deadly airborne fungal infection in developed countries. In order to identify novel antifungal drug targets, we investigated the genome of *A. fumigatus* for genes that are necessary for efficient fungal growth. An artificial *A. fumigatus* diploid strain with one copy of an engineered impala160 transposon from *Fusarium oxysporum* integrated into its genome was used to generate a library of diploid strains by random in vivo transposon mutagenesis. Among 2,386 heterozygous diploid strains screened by parasexual genetics, 1.2% had a copy of the transposable element integrated into a locus

essential for *A. fumigatus* growth. Comparison of genomic sequences flanking impala160 in these mutants with that of the genome of *A. fumigatus* allowed the characterization of 20 previously uncharacterized *A. fumigatus* genes. Among these, homologues of genes essential for *Saccharomyces cerevisiae* growth have been identified, as well as genes that do not share homologues in other fungal species. These results confirm that heterologous transposition using the transposable element impala is a powerful tool for functional genomics in ascomycota and pave the way for defining the complete set of essential genes in *A. fumigatus*, the first step towards a target-based development of new antifungal drugs.

279. Simple Sequence Repeat (SSR) Markers for *Magnaporthe grisea* and Their Integration into an Established Molecular Linkage Map. Claudia Kaye[1] , Joëlle Milazzo[1], Amandine Bordat[1], Sophie Rozenfeld[2], Marc-Henri Lebrun[3] and Didier Tharreau[1].[1]UMR 385, Montpellier France, [2]Bayer CropScience, Evry, France, [3]UMR 1932 INRA-CNRS-Bayer CropScience, Lyon, Fance

Although initially time consuming and expensive to develop, microsatellite markers are robust, easy to assay and easily transferable between different research groups making them excellent markers to act as anchors to integrate maps constructed from different crosses and for locating genes of interest in the genome. We have identified and characterized SSR containing loci in the filamentous ascomycete *Magnaporthe grisea*, the causal agent of rice blast disease. Construction and screening of an SSR enriched library and searching databases were useful tools for finding microsatellites. A total of 49 SSR were tested for their ability to detect polymorphism among six isolates of *M. grisea*. The number of alleles at each locus ranged from one to six when assayed on 3% agarose gels. Inheritance and linkage were determined for 23 polymorphic sites using 58 F1 progeny from the above reference cross. Genetic analysis showed that all the markers segregated in the expected 1:1 ratio. Map positions were determined for all 23 loci. SSR were dispersed on all seven of the chromosomes. New microsatellite markers are now being developed using the sequence of the entire *M. grisea* genome with a goal of saturating the reference map. All possible di, tri and tetra repeats will be used to search the genome for candidate sequences.

280. New modules for versatile PCR-based gene disruption and modification in *Candida albicans*. Susanne Gola, Ronny Martin, Diana Schade, Andrea Walter and Juergen Wendland. Junior Research Group "Growth control of fungal pathogens", Hans-Knoell-Institute and Friedrich-Schiller-University, Jena, Germany.

Gene knockout in the diploid fungus *Candida albicans* requires the disruption of both alleles of the target gene. Recently, an important advance in the functional analysis of *C. albicans* genes was reported by the development of PCR-mediated techniques for gene deletion and GFP-tagging of chromosomal genes (Wilson *et al*., 1999; Gerami-Nejad *et al*., 2001). Here, we present a new set of modules that can serve as templates for the PCR-mediated synthesis of fragments that allow (i) gene disruption (ii) XFP-tagging to the 3`end of the gene and (iii) promotor exchange using regulatable or strong promotors. Due to the modular nature of the plasmids a minimal set of primers is required e.g. for successive gene disruptions. We hope that these plasmids will further facilitate functional gene analysis in*C. albicans*.

281. FRQ-based and FRQ-less oscillators regulate output from the *Neurospora* circadian clock. Zachary Lewis, Alejandro Correa, Andrea Cass, and Deborah Bell-Pedersen. Dept of Biology, Texas A&M Unversity, Texas

Circadian clocks have been examined at the molecular level in organisms ranging from cyanobacteria to humans. These organisms possess endogenous clocks that are synchronized to local time through environmental signals, such as changes in light or temperature. Circadian clocks enable organisms to anticipate changes in the environment and temporally partition biochemical, physiological, and behavioral processes to the appropriate time of the day. In eukaryotic cells, the core clock mechanism involves one or more transcription/translation feedback loops. In the filamentous fungus *Neurospora crassa*, the *frequency (frq), white collar–1 (wc–1), and wc–2* genes comprise one feedback loop. While the contribution of these genes to the time-keeping process is well understood, very little is known about how this molecular clock functions to control the output processes. To identify components of the output pathways in *N. crassa*, we first constructed cDNA microarrays to identify genes that accumulate rhythmic mRNA. We demonstrate that 20% of the genes are clock-controlled, indicating the importance of the clock in the life of the fungus. In addition, we have identified genes that show a circadian oscillation of steady-state mRNA levels in a *frq*-null*(frq^{10})* strain. These data suggest that output pathways from the Neurospora circadian clock are regulated by both FRQ-based and FRQ-less oscillators.

282. Genomic analysis of calcium signalling proteins in *Neurospora crassa*. Alex Zelter[1,2], Oded Yarden[2] and Nick D. Read[1]. [1]Fungal Cell Biology Group, Institute of Cell and Molecular Biology, University of Edinburgh, Scotland. [2]Dept. of Plant Pathology and Microbiology, Hebrew University of Jerusalem, Israel

A considerable body of evidence, primarily from pharmacological studies, indicates that Ca^{2+}-signalling regulates numerous processes in *Neurospora*. These processes include the control of conidial germination, circadian rhythms, tip growth and hyphal branching. Examples include the involvement of Ca^{2+} in suppression of the hyperbranching phenotypes of the mutants *frost* and *spray*, and the fact that genetic or chemical impairment of the catalytic or regulatory subunit of calcineurin (a Ca^{2+}-dependent phosphatase) causes loss of apical dominance and hyperbranching followed by growth arrest. Despite the obvious importance of Ca^{2+}-signalling in *Neurospora*, and in contrast to the situation in budding yeast, only a handful of Ca^{2+}-signalling related genes have been cloned or characterised in *Neurospora* to date. We have made a thorough BLAST analysis of the Ca^{2+}-signalling related proteins encoded in the recently published genome sequence of *Neurospora crassa*. This analysis has identified many of the proteins likely to be necessary for Ca^{2+}-signaling in *Neurospora*. These proteins include previously unknown Ca^{2+}-permiable channels, Ca^{2+}-ATPases, Ca^{2+}/H^{+}-exchangers, Ca^{2+}/Na^{+}-exchangers, phospholipase C proteins and Ca^{2+}/calmodulin binding proteins. Our analysis suggests that *Neurospora*has over 50% more Ca^{2+}-signalling related proteins than budding yeast. These results highlight both the potential importance and apparent complexity of Ca^{2+}-signalling in *Neurospora*. A major challenge for the future will be to develop and adapt the tools necessary to investigate these important proteins experimentally.

283. Functional genomics of *Aspergillus niger* strains. Noël van Peij[1], Hildegard Menke[1], Stefaan Breestraat[1], Rogier Meulenberg[1], Herman Pel[1], Hans van den Hombergh[1], Marco van den Berg[2], Rutger van Rooijen[3], Albert van Ooyen[1], Alard van Dijk[1], Hein Stam[1]. [1]DSM Food Specialties, [2]DSM Anti-Infectives, [3]DSM Bakery-Ingredients, P.O. Box 1, 2600 MA Delft, The Netherlands

The filamentous fungus *Aspergillus niger* is a main microorganism used for enzyme production. Wild-type strains of A. niger have the capacity of secreting large amounts of various enzymes and are potentially suitable host strains for homologous and heterologous gene expression. Classical strain improvement programs as well as defined genetic modifications have been used successfully to improve enzyme productivity. In order to rationalize and speed up the ongoing strain- and process-improvement program, as well as to identify potential new products, DSM sequenced the complete 35.9 Mb genome of *A. niger*. The 7.5-fold coverage random sequencing of carefully selected large insert BACs allowed the assembly of the 8 linkage groups into 19 large so-called supercontigs, each supercontig containing only small sequence gaps. Over 14,000 open reading frames (ORFs) were identified and functionally classified using a combination of specifically trained computer algorithms and manual ORF verification and annotation. This program was one of the largest commercial sequencing projects in Europe. Recently, *A. niger* GeneChips were designed allowing the analysis of the genome-wide transcriptome. The dynamics of *A. niger* strains at the mRNA- and also at the DNA-level can be followed using these DNA chips. Examples of Comparative Genomic Hybridisation and controlled fermentations followed by whole genome transcriptomic analysis will be shown. Especially, the importance of a highly reproducible chip protocol including fermentation sampling, RNA- and chip-processing will be discussed. The *A. niger* genome was sequenced by Gene Alliance (www.gene-alliance.com). The genome was annotated by Biomax Informatics (www.biomax.de). The GeneChips experiments were performed in collaboration with the Microarray Department (www.microarray.nl)

284. Global gene deletion project in *Cryptococcus neoformans*. Jennifer K. Lodge, Maureen J. Donlin and Kim J. Gerik, Saint Louis University, St. Louis, MO USA

Cryptococcus neoformans is an opportunistic fungal pathogen that causes meningioencephalitis in immunocompromised patients, particularly patients with AIDS. Current antifungal agents are inadequate for safe and effective therapy. Over the past three years, a genome project conducted jointly by Stanford University, TIGR and University of Oklahoma has produced >10X coverage of the genome for *C. neoformans* var. *neoformans* as well as cDNA sequences through an EST project. Functional genomics has the potential to accelerate our understanding of pathogenesis in complex organisms. The opportunity exists now to efficiently test the contribution of *C. neoformans* genes to pathogenesis using a mass screening approach employing signature tagged mutagenesis. Each insertion mutant is being tagged with a unique sequence that will allow identification of that mutant within a large pool of mutants. Groups of 48 mutants will be screened together for growth in a mouse model, and mutations in

genes that affect virulence in this competitive assay will be rapidly identified. We have begun a large-scale gene deletion project for *C. neoformans* var. *grubii*. Var. *grubii* is more virulent than var. *neoformans* and therefore is more suitable for virulence screen. In addition, there is limited sequence available for var. *grubii* through Duke University. We have developed bioinformatics tools to help identify genes. We currently have deletions in 14 genes that are homologs of *S. cerevisiae* cell wall genes, including *ALG1*, *CDA1*, *CHS1*, *CRR1*, *CWH8*,*DAN4*, *SMI1*, *YUR1*, *UTR2*, *CHS5*, *ECM4*,*EXG1*, *HOC1*, *SSD1*. These mutants are being analyzed for their in vitro and in vivo phenotypes.

285. Exploring gene expression in *Ustilago maydis*: EST libraries, comparative genomics and microarray hybridization. B.Saville, R. Austin, M. Babu, K. Nugent, N. Sacadura, K. Choffe, N. Provart. Botany, University of Toronto at Mississauga, Mississauga, ON, Canada.

We have created two EST libraries from the causal agent of common smut of corn, *U. maydis*. These libraries are from two cellular stages that vary greatly in physiology and structure, a forced diploid growing as a mycelia on charcoal media plates and the germinating teliospore growing in liquid 1% sucrose and 0.2% casamino acids. Combining the libraries and selecting for contigs followed by the combination of contigs with identical top matches in BLASTX searches reveals 3918 unique contiguous sequences. From sequencing and analysis of the *U. maydis* genome by Bayer a coding capacity of 6700 ORFs was indicated. Thus our EST contigs represent 59% of the coding capacity of the *U. maydis* genome. We have used this substantial coverage in a species by species comparative analysis with genomic and EST sequence databases of pathogenic and non-pathogenic fungi. The results of this analysis will be presented along with a break down of the functional categories of genes discovered in *U. maydis*. The EST identified clones are also being used in the construction of cDNA microarrays for the comparison of gene expression between different cellular states. Data from initial hybridizations with the first generation low-density microarrays will be presented, as well as an update on the construction of higher density arrays.

286. Expression profiling of *b*-mediated gene expression in *Ustilago maydis*. Mario Scherer and Joerg Kaemper, MPI terrestrial Microbiology, Marburg, Germany

The phytopathogenic fungus *Ustilago maydis* has a dimorphic life cycle. For successful infection of its host plant maize two compatible haploid sporidia have to fuse on a leaf surface and form a filamentous dikaryon, which is able to penetrate the plant cuticula and subsequently leads to the fungal proliferation in planta. Filament formation and the subsequent steps in pathogenic development are controlled by the multiallelic *b*-mating type locus encoding the homeodomain proteins bE and bW. bE and bW proteins expressed from different alleles can form a heterodimer, which is thought to regulate the *b*-dependent processes via its function as a transcriptional regulator. Several approaches helped to identify direct and indirect *b*-target genes during the past years, but yet did not reveal a complete view of the biological processes regulated by *b*. Taking advantage of the *U. maydis* genome sequence, a gene chip was designed that allows parallel expression analysis of about 6300 *U. maydis* genes. The gene chip technology was used to compare expression profiles of haploid cells expressing either compatible or incompatible combinations of bE and bW genes under control of two different inducible promoters during an 12 hours time course. We will present data on this genome wide expression analysis of *b*-dependent developmental regulation.

287. BAC by BAC sequencing of the rice blast fungus (*Magnaporthe grisea*) chromosome 7. Michael Thon, Huaqin Pan, Audrey Taro, Douglas Brown, Thomas Mitchell, and Ralph A. Dean. Center for Integrated Fungal Research, Plant Pathology Department, Box 7251, North Carolina State University, Raleigh, NC 27695-7251 USA

Magnaporthe grisea, the causal agent of rice blast disease, is a serious pathological threat to food supplies worldwide. Recent studies have increased our understanding of the biology and molecular determinants of pathogenesis for *M. grisea* and related fungi. These activities provide a starting point and the necessary tools to more thoroughly elucidate the mechanisms involved in host pathogen interactions. Previously, a physical map of the *M. grisea* genome was constructed using a 25X BAC library. Using this BAC library, we are performing large-scale sequence analysis of chromosome 7 using a BAC-by-BAC approach. Selected BAC clones are sequenced to 5X coverage giving an average of 11 contigs per clone. The contigs are being combined with the whole genome shotgun sequence of *M. grisea* in order to reduce the number of gaps and resolve assembly discrepancies. By combining the BAC sequences with the shotgun sequence, we closed approximately 100 gaps. The remaining gaps in chromosome 7 are being closed using a variety of sequencing techniques. We will present results from BAC

sequencing including analyses of gene content and order, EST location, and chromosome-wide synteny with *Neurospora crassa*.

288. Viewing *Aspergillus fumigatus* genomic data via the Web. Jane E Mabey, Michael J Anderson, and David W Denning. Department of Medicine, University of Manchester, Manchester, UK.

The University of Manchester has been funded to manage and curate *A. fumigatus* genomic data. In addition, since the species within the genus *Aspergillus* have recently evolved from each other, this project will enable comparative studies by gathering other *Aspergillus* genomic data into a central data repository - CADRE (the Central*Aspergillus* Data REpository). Using Ensembl, a software system for maintaining and visualising annotated genomes, we have set up a database for housing the annotated *A. fumigatus* mitochondrial sequence received from The Institute for Genomic Research and provided several means of viewing the data. At present, using a Web browser, the sequence contig can be viewed alongside various features (e.g., protein-coding genes, RNA-coding genes and repeats) that have been mapped onto it. For each feature, links are provided to allow the user to retrieve further annotation. For genes, such annotation includes: the locus, a description of the encoded protein's function; transcripts and their sequence; and links to further information regarding translations. Next we will receive 922 kb of sequence centred around the *niaD*locus from the Sanger Institute, which was generated as part of the *A. fumigatus* genome sequencing project. We aim to adapt Ensembl to enable viewing of this sequence and its associated annotation. We are also aiming to provide other web-based tools (e.g., pairwise sequence comparison and community-based annotation) in preparation for the complete *A. fumigatus* genome sequence, which is expected in late 2003.

289. Whole Genome Analysis of Pathogen-host Recognition and Subsequent Responses in the Rice Blast Patho-system. Thomas Mitchell*, Doug Brown*, Nicole Donofrio*, Daniel Ebbole&, Mark Farman$$, Yong-Hwan Lee+, Marc Orbach#, Cari Soderlund#, Guo-Liang Wang^, Rod Wing#, Jin-Rong Xu(), Ralph Dean*. *North Carolina State University Fungal Genomics Laboratory, &Texas A&M University, $$University of Kentucky, +Seoul National University Korea, #University of Arizona, ^Ohio State University, ()Purdue University.

Magnaporthe grisea is the causal agent for rice blast disease and is considered a leading model for studying fungal pathogenesis. A NSF funded project was launched to use genomic approaches to identify and characterize genes that contribute to pathogenicity in the fungus as well as resistance in the host. The goals of this initiative are to characterize putatively secreted proteins from the fungus and identify those that interact with the plant, generate 50,000 random integration fungal mutants to identify genes required for reproduction and pathogenicity, sequence 35,000 ESTs from infected and uninfected rice, analyze six different rice Long SAGE libraries, use over 400 oligo-based interactions microarray chips containing all predicted rice blast genes as well as rice genes identified in the unique set of ESTs, and perform targeted deletions of fungal genes predicted to be involved in pathogenicity. All data generated by this project is centralized in a database called MGOS, and is presented to the public via a web interface designed for researchers to query the data. Here we will present the current status of the project with particular consideration of two key elements. We will highlight the results of the mutant screen and characterization of genes found to be important in pathogenicity. We are also generating oligo-based microarray chips that will be made publicly available. We will present the chip design and status of generation as well as detail the results from chip validation experiments.

290. *Fusarium graminearum* genomics at AAFC. Audrey Saparno, Linda Harris, Robert Watson, Hélène Rocheleau, Anju Koul, Jiro Hattori, Anick DeMoors, Scott Kelso, Laurian Robert, Dave Sprott, Nick Tinker, and Thérèse Ouellet. Eastern Cereal & Oilseed Research Centre, Agriculture & Agri-Food Canada, Ottawa, Ontario, Canada.

We are using a functional genomics approach to explore, at the molecular level, the biological pathways involved in *Fusarium graminearum* (Fg) pathogenicity. A variety of Fg-tissues (macroconidia, mycelia, perithecia), challenged Fg-culture conditions (plant contact, high DON production, growth on cornmeal, starvation/stress treatment) have been used to generate a collection of >6,000 expressed sequence tags (ESTs) which group into ~2800 contigs or singletons. Preliminary data obtained from EST sequencing has already provided hints concerning the gene specificity of each Fg-tissue. We will present the main differences observed between Fg-libraries and some aspects of functional annotation. We will discuss how we have used datamining to select candidate genes suspected to be

essential in the fungal infective process. A 2500 unigene Fg microarray has been produced and will be used for the high-throughput determination of transcriptional profiles.

291. *Phytophthora* Genome Sequence Resources. Brett M. Tyler [1], Felipe Arredondo [1], Howard S. Judelson[2], Ralph A. Dean[3], Mark E. Waugh [4], Jeffry L. Boore[5], Sucheta Tripathy [1] and Bruno W.S. Sobral [1].[1]Virginia Bioinformatics Institute, [2]University of California, Riverside, [3]North Carolina State University, [4]National Center for Genome Resources, NM, [5]DOE Joint Genome Institute, CA.

Plant pathogens of the genus *Phytophthora* cause tens of billions of dollars of damage each year to a huge range of agriculturally and ornamentally important plants. The ornamental, nursery and forestry industries are also heavily affected. These pathogens outwardly resemble fungi, but are in fact more closely related to brown algae such as kelp. To create a resource to assist in identifying genes involved in pathogenicity and targets for control measures, we have developed a comprehensive survey of gene sequences of two key *Phytophthora* species that are expressed during growth, spread and infection. The species are *P. sojae*, which infects soybean, and *P. infestans*, which infects potato and tomato. We have cloned and sequenced over 30,000 messenger RNAs from *P. sojae* and over 10,000 from *P. infestans* from axenic culture and from infection sites. We have also commenced an 8X shotgun sequencing of the *P. sojae* genome (62 Mb) and a 4X sequence of the genome of the forest pathogen *Phytophthora ramorum*. Progress on these genome sequences will be reported. For further information see https://xgi.ncgr.org/pgc and www.vbi.vt.edu.

292. Fungal genes expressed during plant disease development in the *Fusarium graminearum*/wheat interaction. R.S. Goswami[1], F. Trail[2], J.R. Xu [3], H.C. Kistler [4]. [1]Dept. Plant Pathology, Univ. of Minnesota, St. Paul, MN 55108. [2] Dept. Botany and Plant Pathology, Michigan State Univ., E. Lansing, MI 48824. [3]Dept. Botany and Plant Pathology, Purdue Univ., West Lafayette, IN. [4]USDA-CDL, St. Paul, MN

Fusarium graminearum the causal agent of wheat scab disease is a species complex comprised of strains belonging to at least eight phylogenetically distinct lineages that can differ significantly in their aggressiveness on wheat and also in the type and amount of mycotoxin they produce. Based on differences in aggressiveness we selected two strains with high (NRRL 31084) and low (NRRL 28303) virulence for genomic studies. cDNA libraries were created by suppression subtractive hybridization to compare mRNA populations from wheat heads inoculated with them in order to identify genes specific to each interaction. EST sequences from both the forward and reverse libraries revealed marked differences in gene expression among strains during pathogenesis. Several of them had matches with sequences from other fungi indicating that they were *Fusarium* genes expressed *in planta*. Another subtracted cDNA library also has been constructed using wheat inoculated with NRRL 31084 and mock inoculated wheat heads to further characterize fungal genes expressed during the disease interaction. Ultimately, we anticipate that this information will be vital for identification, isolation and functional analysis of genes related to pathogenicity.

293. Analysis of telomere-associated sequences in *Magnaporthe grisea*. Cathyrn Richardson, Motoaki Kusaba, Venu Gopal-Puram, Weixi Li, Sherri Schwartz Chuck Staben, Chris Schardl and Mark Farman. Department of Plant Pathology, University of Kentucky, Lexington, KY

In many eukaryotic microbes, subtelomere regions harbor genes with important roles in niche adaptation, with frequent recombination in these locations producing variation that fuels adaptation. Some *M. grisea* telomeres are known to contain avirulence genes, suggesting that telomere-associated genes may contribute to pathogenic adaptation in this fungus. To test this hypothesis, we are cloning and sequencing the *M. grisea* telomeres. This will also enable extension of the recently-obtained genome sequence out to the chromosome tips. We have developed a ligation-anchored PCR-based method that targets amplification of subtelomeric regions of the genome. Our strategy effectively enriches for telomere-proximal sequences and promotes the rapid analysis of sequences associated with specific chromosome ends. Amplicons generated by this method have been used as probes to identify cosmids containing longer telomere-associated sequences in genomic libraries of *M. grisea*. These cosmids are currently being sequenced. In addition, we have isolated and sequenced cosmids containing members of a telomere-linked helicase gene family. We will present an overview of *M. grisea* telomere organization and will also demonstrate how the telomere sequences obtained using our targeted approach connect to the current *M. grisea* genome assembly.

294. Generation of a wheat leaf rust, *Puccinia triticina*, EST database from stage-specific cDNA libraries. G.G. Hu, R. Linning, B. McCallum, T. Banks, S. Cloutier, M. Jordan, C. Matsalla, J. Schein, Y. Butterfield, S. Jones, M. Marra and G. Bakkeren. Agriculture and Agri-Food Canada: G.G.H, R.L., G.B., Highway 97, Summerland, BC; B.M., T.B., S.C., M.J., 195 Dafoe Rd., Winnipeg, MB; C.M., 107 Science Place, Saskatoon, SK; J.S., Y.B., S.J., M.M., Genome Sciences Centre, 600 W. 10th Avenue, Vancouver, BC.

We have optimized methods for the construction of cDNA libraries from small amounts of mRNA representing several developmental stages of the wheat leaf rust fungus, *Puccinia triticina*. These stages included urediniospores, urediniospores germinated on water or plant extract, and interactive, appressorial and haustorial stages on a compatible host. These transcript populations were subjected to a variety of treatments such as full-length cDNA production, subtractive and normalizing hybridizations, and size selection methods combined with PCR amplification or traditional cloning using adapters. We sequenced 14,000 clones from which a non-redundant unigene set of over 3,000 ESTs was assembled. Ten percent exhibit homology via BLAST to known genes in public databases while an additional 10% match other ESTs of unknown function in a custom database of collected, public fungal sequences. Interactive libraries have been subtracted for wheat sequences *in silico* using a wheat EST collection and public sequences. Information on gene categories will be presented. This research has been funded by the Agriculture & Agri-Food Canada 'Crop Genomics Initiative'.

295. An ensemble method for identifying regulatory circuits with special reference to the *qa* gene cluster of *Neurospora crassa*. D. Battogtokh, D. K. Asch, M.E. Case, J. Arnold, and H.-B. Schuttler. Genetics, University of Georgia, Athens GA.

A chemical reaction network for the regulation of the quinic acid (*qa*) gene cluster of *Neurospora crassa* is proposed. An efficient Monte Carlo method for walking through the parameter space of possible chemical reaction networks is developed to identify an ensemble of deterministic kinetics models with rate constants consistent with RNA and protein profiling data. This method was successful in identifying a model ensemble fitting available RNA profiling data on the *qa* gene cluster.

296. Genome sequencing of *Aspergillus oryzae*. M. Machida[1], M. Sano[1], K. Asai[1], T. Kin[1], H. Nagasaki[1], T. Komori[2], T. Tanaka[3], R. Igarashi[3], O. Akita[4], Y. Kashiwagi[5], K. Gomi[6], K. Abe[6], M. Takeuchi[7], T. Kobayashi[8], H. Horiuchi[9], K. Kitamoto[9] ([1]Natl. Inst. Advanced Ind. Sci. Tech.,[2]INTEC Web and Genome Informatics, [3]Natl. Inst. Tech. Eval,[4]Natl. Res. Inst. Brewing, [5]Natl. Food Res. Inst.,[6]Tohoku Univ., [7]Tokyo Univ. Agric. Technol.,[8]Nagoya Univ., [9]Univ. Tokyo)

The whole genome sequencing project for *Aspergillus oryzae* launched in August 2001. Because there are no genetic maps of the *A. oryzae* availble, the sequencing is being done mainly by a whole genome shotgun sequencing approach in combination with some chromosome specific shotgun sequencing. A rough draft of the *A. oryzae* genome was completed in January 2002 by accumulating sequences of approximately 6X depth of coverage after 600,000 sequence reactions. Tentatively, the total genome size of *A. oryzae* is estimated to be 37 Mb with 954 contigs and 36 supercontigs. The computational gene finding by GeneDecoder (Asai et al., http://www.cbrc.jp/) predicts presence of over 13,000 genes including ca. 1,700 for membrane proteins, ca. 380 for transcription factors, ca. 160 for translation factors, and ca. 70 for protein secretory pathways besides abundant genes for hydrolytic enzymes. Approximately 40% of genes are speculated to have introns. The completely fixed sequences will be published for each chromosome without significant delay.

297. Population genetic variation in genome-wide gene expression in wine yeast. Jeffrey Townsend, Duccio Cavalieri and Daniel Hartl Plant and Microbial Bio, University of California, Berkeley, Berkeley, CA and Harvard University, Cambridge MA.

Genome-wide transcriptional profiling allows assessment of heterozygosity for alleles showing quantitative variation in gene expression in natural populations. We have used DNA microarray analysis to study global pattern of transcription in strains of Saccharomyces cerevisiae isolated from wine grapes in a Tuscan vineyard, along with the diploid progeny obtained after sporulation. One parental strain shows 2:2 segregation (heterozygosity) for a morphological phenotype observed as colonies with a ridged surface resembling a filigree. Global expression

analysis of the progeny with the filigreed and smooth colony phenotypes revealed a greater than 2-fold difference in transcription for 378 genes (6% of the genome). A large number of the abundantly expressed genes function in pathways of amino acid biosynthesis (particularly methionine) and sulfur or nitrogen assimilation, whereas many of the meagerly expressed genes are amino acid permeases. These wholesale changes in amino acid metabolism segregate as a suite of traits resulting from a single gene or small number of genes. Other comparisons show consistent strain to strain variation in gene expression. We can conclude that natural vineyard populations of *Saccharomyces* can harbor alleles that cause massive alterations in the global patterns of gene expression. This work represents the first demonstration of global gene expression variation in natural populations of any organism, and argues for an enhanced consideration of the role of gene expression variation in the evolution of new traits.

298. Isolation and characterization of the methylated component of the *Neurospora crassa* genome. Eric U. Selker[1], Nikolaos Tountas[2], Sally Cross[2, 3], Brian S. Margolin , Jonathan G. Murphy , Adrian P. Bird[2] and Michael Freitag[1] ([1]University of Oregon, Eugene, USA; [2]Univ. of Edinburgh, UK; [3]Western General Hospital, Edinburgh, UK; [4]Univ. of California San Franciso, USA).

The filamentous fungus *Neurospora crassa* serves as a model organism to elucidate the function and control of eukaryotic DNA methylation. Few natural methylated sequences have been identified in Neurospora, however. We report the isolation and characterization of a representative sample of the methylated component of the Neurospora genome. We used the methylated-DNA-binding domain of mammalian MeCP2 as an affinity reagent to enrich for methylated DNA, cloned fragments from methylated fractions and characterized them by differential hybridization, sequence analyses and Southern hybridizations. All methylated fragments showed evidence of RIP, suggesting that most, if not all, vegetative DNA methylation in Neurospora occurs in regions previously subjected to RIP or in regions resembling RIP-mutated DNA. Most methylated fragments were related to mobile elements, which were found both clustered in repeat-rich centromeric regions or dispersed throughout euchromatin. In addition to homologues of four previously described transposon relics, we identified seven groups of transposon relics previously unknown from Neurospora, including gypsy-type retroelements and several DNA-type transposons of the Tc1, hAT and pogo superfamilies. Finally, we used the genome sequence of Neurospora to identify relics of RIP and tested them for DNA methylation. Almost all (19/20) of these sequences were found to be methylated. (Supported by NIH grant GM35690 and a Senior International Fellowship from the Fogarty International Center to E.U.S.).

299. Cloning of telomeric regions from *Neurospora crassa* wild-type strains Oak Ridge and Mauriceville. Cheng Wu, Julie Mitchell, Mark L. Farman* and Matthew S. Sachs. Oregon Health and Science University, Beaverton, OR 97006 *University of Kentucky, Lexington, KY 40546

Linear eukaryotic chromosomes terminate in simple sequence repeats called telomeres. New telomere repeats, typically 5' TTAGGG 3', are added to the existing chromosome ends to guard against the loss of DNA during replication. In fungi and other eukaryotic microbes, regions near telomeres are highly variable and may be rich in genes for ecological adaptation. Telomeres are poorly represented in the draft genome sequence database for *Neurospora crassa*. By characterizing clones for *N. crassa* telomeres and subtelomeric regions, we will be able to assess the functional and evolutionary significance of these regions and complete the genome sequence. To clone *N. crassa* telomeric regions, isolated chromosomal DNA fragments with polished ends were first ligated to linearized Bluescript vector. The ligated DNA was cut with a cohesive-end restriction enzyme, recircularized and transformed into *Escherichia coli* XL-10 cells. Recombinant colonies containing telomeres were then identified using a ^{32}P-labeled telomere probe. Our goal is to isolate and sequence the 14 telomeric regions of two different wild-type *N. crassa* strains Oak Ridge and Mauriceville. So far 10 apparently unique telomeres from the Oak Ridge strain and 5 apparently unique telomeres from the Mauriceville strain have been captured. Comparison of subtelomeric regions between strains will improve understanding of the pathways of genome evolution.

300. The Fungal Genome Initiative. Li-Jun Ma, James Galagan, Sarah Calvo, Cydney Nielsen, Tim Elkins, Robert Barrett, Mary Wong, Eric Lander, Chad Nusbaum, and Bruce Birren. Whitehead Institute/MIT Center for Genome Research, Cambridge, USA

The Fungal Genome Initiative was developed to sequence and analyze fungal genomes at various evolutionary distances within the Fungal Kingdom. The goal of the project is to utilize the power of comparative genomics to gain deeper understanding of fungal evolution, genetics, physiology, developmental regulation, and especially to

understand the basis of fungal pathogenecity. Further, this kingdom-wide comparative study can lead us to understand the evolution of Eukaryotic genes, chromosomes, and regulatory and biochemical pathways. The FGI steering committee selected an initial list of important fungal sequencing targets. In 2002 a review by the National Human Genome Research Institute established the FGI organisms as a High Priority for genome sequencing. In the next year, seven genomes will be sequenced at the Whitehead for public release. These are *Aspergillus nidulans, Cryptococcus neoformans*,serotype A, *Coccidiodes immitis, Pneumocystis carinii* (human and murine), *Rhizopus arrhizus, Coprinus cinereus, Ustilago maydis*. We will describe the current status and plans for this project.

301. Improving the *Neurospora crassa* Genome Sequence. Cydney Nielsen[1], James Galagan[1], Li-Jun Ma[1], Sarah Calvo[1], Jonathan Butler[1], Dayong Qui[1], David Jaffe[1], Seth Purcell[1], William FitzHugh[1], Robert Barrett[1], Tony Rachupka[1], Michael FitzGerald[1], Nicole Stange-Thomann[1], Xiaoping Yang[1], Peter Ianakiev[1], John Major[1], Michael Frietag[5] Asha Kamat[1], Matthew Sachs[2], Jak Kinsey[3], Chuck Staben[4], Eric Lander[1], Chad Nusbaum[1], and Bruce Birren[1]. [1]Whitehead Institute Center for Genome Research, Nine Cambridge Center, Cambridge, MA, USA.[2]Department of Biochemistry and Molecular Biology, OGI School of Science and Engineering, Oregon Health & Science University, Beaverton, OR, USA.[3]Department of Microbiology, University of Kansas Medical School, Kansas City, KS, USA. [4]T.H. Morgan School of Biological Sciences, University of Kentucky, Lexington, KY, USA. [5]University of Oregon, Eugene, USA

Efforts are underway to produce a complete sequence of the *Neurospora crassa* genome. Working towards this goal, we generated a high-quality draft sequence of this >40 Mb genome that is available for download and query (*Neurospora crassa* Database Release 3). Here we report significant improvements to the draft sequence achieved through the addition of jumping clone sequences and the application of various gap closure techniques. The current assembly consists of 958 sequence contigs, comprising 38.9 Mb, ordered and oriented into 163 scaffolds by links arising from paired-end reads. Jumping clone links have improved the long-range contiguity of scaffolds, such that 50 % of all bases are contained in a scaffold of at least 1.5 Mb. Notably, the assembly now contains a >2 Mb scaffold spanning the centromere of chromosome VII. Placement of genetic markers has allowed us to anchor the vast majority of the assembly (95%) to the Neurospora genetic map. The majority of sequence gaps are present in sub-clones that provide the templates for additional sequencing needed for genome closure. Progress of ongoing finishing efforts will be reported.

302. Construction and preservation of *Monascus purpureus* genetic libraries. Li Ling Liaw; Gwo Fang Yuan; Wen Shen Chu; Chao Zong Lee and Chii Cherng. Liao Bioresource Collection and Research Center of Food Industry Research and Development Institute, Hsinchu, Taiwan

Genetic library is the basal of the genetic studies. *Monascus purpureus* BCRC 38072 is a strain with the ability of producing hypocholesterolemic agent –monacolin K. The genome size of M. purpureus BCRC 38072 was about 35 +1 mb according to PFGE analysis. We have constructed and preserved nine *M. purpureus* libraries including one BAC library, six cDNA libraries, one cosmid library, and one fosmid library. The BAC library has an average insert size of 131 kb and a total of 12,672 clones, which covered over ten times of the genome size. Average insert sizes of 41.5 and 39.3 kb respectively were constructed in the fosmid and cosmid libraries. The average insert size of six cDNA libraries ranged from 1.1 to 2.0 kb, and 6912, 4224, 11520, 11520, 18432, 19584 clones of these libraries were stored in the collection system of BCRC. The long-term and safe preservation method of the genetic libraries has been established. Duplicated copies of these cosmid, fosmid, cDNA and BAC libraries mentioned above were each prepared in 11 to 51 micro-plates of 384 wells. The standard operation practices of authentic methods for genetic resources such as DNA sequencing, restriction enzyme mapping and PCR fragment analysis were established. We performed the phage screen and survival test of ten 384-plates of BAC library. No phage contamination was found in BAC clones screened. After six months preservation, the survival rate of the tested BAC clones is 100%.

303. Whole Genome Annotation. Sarah Calvo, James Galagan, Bruce Birren. Whitehead Institute/MIT Center for Genome Research, Cambridge, MA.

With the annotated genomes of Neurospora and Magnaporthe currently available, and eight more whole genome sequence projects scheduled for this year, a whole new array of data is available for fungal genetics and molecular biology. Automated annotation produces a first pass set of gene predictions from the genome assembly. Since these

genes are the basis for further investigation, it's important for researchers to understand the creation and validation processes that underlie these predictions.

We present the methodology of the Calhoun Automated Annotation System used to annotate the fungal genomes sequenced at the Whitehead Institute. We will discuss the different kinds of information available to aid in gene prediction, the problems that arise from conflicts between these data, and the power and limitations of the current state of the art in automated annotation.

497. Re-annotation of ORF start and stop sites in *S. cerevisiae* based on comparisons of orthologous ORFs from five other *Saccharomyces* species. Christie, K. R.[1], Sethuraman, A.[1], Balakrishnan, R.[1], Dolinski, K.[1], Dwight, S. S.[1], Fisk, D. G.[1], Hong, E. L.[1], Theesfeld, C. L.[1], Kamvysselis, M.[2], Paul Cliften, P.[3], Costanzo, M. C.[1], Engel, S. R.[1], Issel-Tarver, L.[1], Dong, S.[1], Weng, S.[1], Johnston, M.[3], Lander, E.[2], Botstein, D.[1], and Cherry, J. M.[1]. [1] Department of Genetics, Stanford University, Stanford, CA. [2] Whitehead Institute for Biomedical Research, MIT, Cambridge, MA. [3] Department of Genetics, Washington University School of Medicine, St. Louis, MO.

Since the publication of the genome sequence of *S. cerevisiae* in 1996, corrections have been ongoing to improve the accuracy of the sequence and the annotations of open reading frames (ORFs) and other sequence features. While most of these changes have been made on a gene by gene basis, the availability of genome sequences for five *Saccharomyces* species (*S. paradoxus*, *S. mikatae*, *S. bayanus*, *S. kluyveri*, and *S. castelli*) from groups at MIT and Washington University allowed the *Saccharomyces* Genome Database (SGD) to make a large scale comparison of orthologous ORFs in these species and re-evaluate ORF boundaries in *Saccharomyces cerevisiae*. Using the Fungal Alignment viewer and Synteny Viewer (links under Comparison Resources on SGD locus pages), users may view protein and DNA sequence from the other *Saccharomyces* species. For 402 of the ORFs considered, there were differences in the length of the one or more ORFs relative to other species at either or both the amino-terminal and carboxyl-terminal ends of the predicted protein sequence. Careful comparison of the DNA sequences, predicted protein sequences, and available literature for these ORFs has allowed us to conclude that changes should be made to the ORF start and/or stop sites for 104 ORFs. A further 161 changes were suggested, but require additional sequencing of *S. cerevisiae* S288C for confirmation. Any changes made will be documented in Locus History notes and a paper will be published detailing the changes to be made. Thus, comparison of *S. cerevisiae* with three closely related and two more distantly related *Saccharomyces* species has allowed us to improve the ORF annotations in SGD.

Host Parasite Interactions Abstracts

304. Functional analysis of Pls1p, a tetraspanin involved in pathogenicity of *Magnaporthe grisea* on rice. Mathieu Gourgues[1], Christophe Fargeix[1], Fabien Cottier[1], Joaquim Cots[1], Marie Pascale Latorse[2] and *Marc-Henri Lebrun[1]. [1]CNRS-Bayer CropScience, Lyon, France.[2]Fungicides Biology Dpt, Bayer CropScience, Lyon, France

PLS1 is required for pathogenicity on rice of the fungus *Magnaporthe grisea*. pls1- mutant produces melanised appressoria with normal turgor that fail to penetrate into host leaf. Pls1p is a 225 amino acids membrane protein related to animal tetraspanins. These proteins participate to membrane signaling complexes involved the control of cell adhesion, differentiation or motility. This type of signaling pathways could be involved in appressorial mediated penetration. We showed that Pls1p was only expressed in appressoria differentiated on leaves or artificial surfaces. Pls1p differential expression is regulated at the post-transcriptional level, since *PLS1* mRNA was detected in all fungal cells. Deletion analysis of PLS1 UTR's showed that the controlling sequences are located in the 5'UTR. We constructed a Gfp-Pls1p fusion protein that is functional since it complements the pls1- deletion mutant. This fusion protein was localised in appressorial plasma membrane and vacuoles. The Gfp-pls1p fusion protein was also

expressed under the control of the constitutive *MPG1* promoter in mycelium without visible phenotypes. Transformants over-expressing Pls1p are currently used for immuno-precipitation experiments. Domains of Pls1p were modified by site directed mutagenesis and assessed by complementation of the pls1- mutant. The small domain between TM2 and TM3 is essential for Pls1p function. Since this domain is likely to be cytoplasmic, it may be required for the interaction between Pls1p and cytoplasmic proteins. A model of*PLS1* function during fungal penetration of rice leaves will be presented.

305. cAMP signaling pathway positively regulates germination and infectious growth in*Colletotrichum lagenarium*. Junko Yamauchi, Kenichi Komeda, Naoyuki Takayanagi, Yoshitaka Takano, and Tetsuro Okuno. Agriculture, Kyoto University, Kyoto, Japan.

Colletotrichum lagenarium, the casual agent of cucumber anthracnose, develops a specialized infection structure called an appressorium to invade host plants. Functional analysis of the regulatory subunit gene of a cAMP-dependent protein kinase (PKA) has shown that hyper-activation of PKA impaired growth, conidiation and appressorium function in this fungus. To investigate the effect of inactivation of cAMP-PKA pathway, the adenylate cyclase gene (*CAC1*) and the PKA catalytic subunit gene (*CPK1*) of *C.lagenarium*were functionally characterized. The *CAC1* and *CPK1* genes were isolated using a PCR-based strategy with degenerate primers. The targeted disrupted mutants of each gene were generated. Both the *cac1* and *cpk1* mutants lacked pathogenicity to the host plant cucumber, suggesting these genes are essential for fungal pathogenicity. Conidia of these mutants hardly germinated on both the glass and host plant surfaces. Germination of the *cac1*mutants was restored by the addition of cAMP, whereas that of the *cpk1* mutants was not. These results indicate that the cAMP-Cpk1 pathway plays important roles for germination in *C. lagenarium*. Furthermore, in contrast to the wild-type strain, when mycelia of the*cac1* and *cpk1* mutants were inoculated on wounded sites of cucumber leaves, they failed to form lesions, suggesting *CAC1* and *CPK1* are necessary for infectious growth inside the host plant. These results indicate that cAMP signaling positively regulates germination and infectious growth. The Cmk1 MAP kinase has also been shown to regulate germination and infectious growth in *C. lagenarium*, suggesting that the Cmk1 MAP kinase and cAMP signaling pathways coordinately regulate germination and infectious growth.

306. Thermotolerance conferred to a broad plant host range by an endophytic fungus isolated from a thermotolerant plant. Joan Henson, Rusty Rodriguez, and Regina Redman. Department of Microbiology, Montana State University, Bozeman, MT and USGS, Seattle, WA.

Adaptation of plants to selective pressure is considered to be regulated by the plant genome. However, recent studies indicate that fitness benefits conferred by mutualistic fungi also contribute to plant adaptation. The survival of both a plant host (*Dichanthelium lanuginosum*) and a fungal endophyte (*Curvularia* sp.) in geothermal soils is dependent on symbiotically conferred thermotolerance. This fungus also asymptomatically colonizes and confers thermal and/or drought tolerance to nonthermal-adapted eudicots and monocots. The symbiotic communication responsible for thermal and drought tolerance is different, since at least one host becomes drought, but not heat tolerant, when colonized by the fungus. Possible mechanisms of conferred drought and thermotolerance will be discussed.

307. High affinity phosphate uptake in ectomycorrhizal *Tricholoma* species. Erika Kothe, Katrin Krause, Doreen Müller. Microbiology, FSU Jena, Germany.

Phosphate uptake and delivery is one essential function of the fungus in ectomycorrhizal symbiosis since woodland soils generally are limited in phosphate. The phosphate is mobilized by short range substrate acidification and then transported as polyphosphate from the substrate hyphae to the plant and into the vascular tissues. For phosphate uptake into the fungal hyphae, high affinity phosphate transporters are responsible. In *T. vaccinum* a gene hybridizing to the *N. crassa* proton symporter of the high affinity phosphate transporter family was shown. The use of proton as symported ion is limited to acidic environments which allow easy access to protons. This is in accordance with medium acidification by *T. vaccinum* under neutral or alkaline conditions. For *T. terreum* found on neutral to alkaline soils a different gene hybridizing to the *N. crassa* sodium symporter could be shown allowing this fungus access to phosphate independent of initial medium pH. For both transporters expression was shown to be higher under phosphorus starvation with a basal expression level of the sodium symporter in *T. terreum* at high phosphate concentrations.

308. *APH1*, a gene encoding a putative methyltransferase, is involved in appressorial penetration into the host plant by *Colletotrichum lagenarium*. Naoyuki Takayanagi, Yoshitaka Takano, Akiko Kimura, and Tetsuro Okuno. Department of Agriculture, Kyoto University, Kyoto, Japan.

Colletotrichum lagenarium, the casual agent of cucumber anthracnose, invades into the host plant using specialized infection structures called appressoria. A pathogenicity-deficient mutant KE51 was isolated by restriction enzyme-mediated DNA integration (REMI) mutagenesis. Molecular analysis of the mutant KE51 identified the *APH1* gene as a gene disrupted by the plasmid insertion. The *aph1* knockout mutants were generated by target gene replacement. The *aph1* mutants showed significant reduction in pathogenicity the same as the original REMI mutant KE51. This indicates that the *APH1* gene is required for fungal pathogenicity in *C. lagenarium* and that the plasmid insertion into*APH1* is responsible for the reduced pathogenicity of KE51. Deduced amino acid sequence of Aph1 has a high homology with the methyltransferase-related proteins of other organisms. Aph1 conserved motifs commonly found in AdoMet-dependent methyltransferases. The *aph1* mutants exhibited the phenotype similar to that of the wild type in colony growth, conidiation, conidial germination, and appressorium formation. By inoculation through wounded sites, the *aph1* mutants formed lesions like the wild-type strain, suggesting that *APH1* is not essential for infectious growth inside the host plant. However, the *aph1* mutants showed a severe reduction in penetration into the host plant, indicating that*APH1* is involved in a penetration step. In contrast, the *aph1* mutants efficiently penetrated into cellulose membranes like the wild type. These data strongly suggest that a putative methyltransferase encoded by *APH1* is specifically involved in appressorial penetration into the host plant.

309. The *SNF1* gene is required for appressorium maturation and fungal pathogenicity in*Colletotrichum lagenarium*. Makoto Asakura, Naoyuki Takayanagi, Yoshitaka Takano, and Tetsuro Okuno. Department of Agriculture, Kyoto University, Kyoto, Japan.

Colletotrichum lagenarium is the causal agent of cucumber anthracnose disease. *C. lagenarium* forms specialized infection structures called appressoria under poor nutrient conditions such as on host plant and artificial glass surfaces. Functional analysis of a peroxisomal biogenesis gene *PEX6* (*ClaPEX6*) demonstrated that peroxisomal metabolic function is required for maturation of appressoria in this fungus. It has been shown that Snf1 protein kinases play a role as a metabolic sensor in several organisms. To assess the relation between appressorium formation and metabolic regulation, we investigated roles of*SNF1* (*ClaSNF1*) in *C. lagenarium*. *ClaSNF1* was isolated using a PCR-based screen with degenerate oligonucleotide primers and the nucleotide sequence of this gene was determined. *ClaSNF1* encodes a protein of 738 amino acids and exhibited significant homology to Snf1 proteins in other organisms. The *clasnf1* knockout mutants were generated by target gene disruption. The *clasnf1* mutants exhibited reduction in vegetative growth on nutrient media. The mutants lacked pathogenicity to the host plant, indicating essential roles of *ClaSNF1* for fungal pathogenicity. The mutants germinated effectively on the glass surface, and germ tubes differentiated into swollen appressoria. However, appressoria formed by the *clasnf1* mutants were relatively small and less melanized compared with those formed by the wild type, indicating that *ClaSNF1* is required for appressorium maturation. Our findings that *ClaSNF1* and *ClaPEX6* are commonly required for appressorium maturation suggest a possibility of involvement of*ClaSNF1* in regulation of peroxisomal metabolism.

310. Novel developmental processes associated with infection of roots by the rice blast fungus. Ane Sesma and Anne E. Osbourn. The Sainsbury Laboratory, John Innes Center, Colney Lane, Norwich NR4 7UH, U.K

Although the rice blast fungus *Magnaporthe grisea* is traditionally regarded as a foliar pathogen, this fungus can also cause disease symptoms on cereal roots. *M. grisea* is closely related to other pathogenic (*M. poae*, *M. rhizophila* and*Gaeumannomyces graminis*) and non-pathogenic (*Phialophora* spp.) root-infecting fungi. Many of the genes that are required for pathogenesis-related development during infection of leaves are dispensable for root infection. Remarkably, *M. grisea* is capable of undergoing a range of developmental processes that are typical of root pathogens and forms microsclerotia, runner hyphae and hyphopodia. Bulbous swollen hyphae can be observed within the root cortex, and the fungus progresses through the cortex to invade the stele. Infection studies with GFP-expressing transformants indicate that *M. grisea* can spread from the roots to the leaves and produce lesions, suggesting that the soil may be a source of inoculum for the establishment of rice blast disease in the field. Mutants that are defective in infection of rice roots have been isolated following *Agrobacterium* -mediated random insertional mutagenesis and new genes required for root colonisation are being characterised. This research is

financially supported by a Marie Curie Fellowship of the European Community. The Sainsbury Laboratory is supported by The Gastby Charitable Foundation.

311. Identification and characterisation of five hydrophobin genes in *Fusarium verticillioides*. Uta U Fuchs [1] and James A Sweigard[2]. [1]Department of Plant and Soil Sciences, University of Delaware, Newark, DE, USA [2]DuPont, Crop Genetics, Newark, DE, USA

We have identified five hydrophobin genes in *Fusarium verticillioides*, a major corn pathogen. Hydrophobins are small, secreted fungal proteins with a characteristic spacing of eight cysteines. The hydrophobin genes were identified from cDNA libraries and from genomic sequencing efforts. FvHYD1 and FvHYD2, encoding class I hydrophobins, were both highly expressed in liquid mycelial cultures. Their potential gene products, FvHyd1p and FvHyd2p, are 80% similar. Class II hydrophobins FvHYD4 and FvHYD5 were obtained from transcripts in microconidia and from genomic sequence, respectively. FvHYD3 messages were found with low abundance in a range of culture conditions. Compared to other hydrophobins, FvHyd3p has only four amino acids between the third and fourth cysteine whereas a 17-39 amino acid loop is common in class I hydrophobins and an 11 amino acid loop in class II hydrophobins. Null mutants were created by gene replacement for each of the genes. None of the mutants showed phenotypic differences from the wild-type in the rate of radial growth and in the number of conidia produced on solid medium, as well as in the amount of disease caused in a corn seedling infection assay. Since FvHYD1 and FvHYD2 are highly expressed and since FvHyd1p and FvHyd2p are very similar, we speculate that either gene can compensate for the absence of the other and hypothesize that a double mutant missing both genes will show a measurable phenotype in growth and infection assays.

312. A unique binuclear zinc transcription factor regulates the *Fusarium solani* cytochrome P450 virulence gene responsible for detoxification of the host's phytoalexin. David Straney[1], Reynold Tan[1], Lydia Rivera[2], and Ever Ponciano[1]. [1] Dept. Cell Biol. & Mol. Genetics, University of Maryland, USA, [2] University of Puerto Rico, USA.

Fusarium solani (teliomorph:*Nectria haematococca* MPVI) induces several virulence traits upon exposure to pisatin, the isoflavonoid phytoalexin produced by its host plant. One response is the induced transcription of a cytochrome P450 (PDA1) that detoxifies pisatin. Although regulators of xenobiotic detoxification-associated P450s belong to conserved families of nuclear receptors in metazoans, these families are absent in fungi. Do fungi regulate their detoxification-associated P450 genes in a parallel manner? We have identified a pisatin-responsive element in the promoter for the *Fusarium* cytochrome P450 gene and cloned a gene encoding a binuclear zinc transcription factor that binds this element. Use of RNAi to suppress expression of the native transcription factor abolishes pisatin induction of the native cytochrome P450 gene. Further, transfer of the *Fusarium* transcription factor and pisatin-responsive elements into a pisatin-naive heterologous fungal system confers strong pisatin-responsive expression of a reporter gene linked to the binding elements. High specificity in this heterologous pisatin-response suggests that the cloned transcription factor is acting as a receptor for pisatin. Such mode of action would provide a model for studying fungal perception of host plants through chemical cues.

313. Analysis of differential gene expression during the symbiosis between*Neotyphodium lolii* and perennial ryegrass. Richard D. Johnson, Shalome A. Campbell and Gregory T. Bryan. AgResearch Ltd., Palmerston North, New Zealand.

Neotyphodium lolii is a fungal endophyte that lives entirely within the intercellular spaces of its grass host, perennial ryegrass. The association is mutually beneficial since the endophyte confers a number of biotic and abiotic advantages to the host, including enhanced plant growth, protection from certain mammalian and insect herbivores, enhanced resistance to nematodes and some fungal pathogens and in some associations enhanced drought tolerance. We are interested in studying the molecular basis of this important symbiosis and hope to identify genes which are important in both its establishment and maintenance, some of which could be fundamental to understanding how plant-fungal interactions, particularly evasion of host defences, are regulated. Using Suppressive Subtractive Hybridisation (SSH), we have generated subtractive cDNA libraries from *N. lolii* infected and un-infected perennial ryegrass. Genes up-regulated or down-regulated during these symbioses are currently being identified. In a parallel approach, proteins which appear to be up or down regulated between the infected and un-infected state have been isolated by 2D-gel electrophoresis and subjected to MALDI-TOF mass spectrometry for identification. Crucial to

this, is the generation of a fungal sequence database comprising random genomic sequences, SSH sequence data, and fungal expressed sequence tags derived from liquid culture. *Funding by FRST (NZ) is acknowledged*.

314. Cloning of a gene encoding an Alt a 1 isoallergen differentially expressed by the necrotrophic fungus *Alternaria brassicicola* during Arabidopsis infection. Robert A. Cramer, Juan Wang, and Christopher B. Lawrence. Colorado State University, Department of Bioag. Sciences Fort Collins, Colorado 80523-1170

Species of Alternaria are considered some of the most important fungi responsible for allergenic morbidity in humans. The Alternaria protein that elicits the most intense allergic reaction in humans is Alt a 1, yet, no known biological function has been identified for this protein. In this study, suppression subtractive hybridization and virtual northern blots were used to identify and characterize an Alt a 1 homolog in the phytopathogenic fungus *Alternaria brassicicola*. RNA was extracted from *A. brassicicola* spores germinated in water and on the leaf surface of the Arabidopsis ecotype Landsberg for 24 hours, and used to create cDNA using PCR. Double stranded cDNA was then used in suppression subtractive hybridization to identify differentially expressed genes. Messenger RNA transcript levels were assessed by virtual northern blots. A sequence with significant homology (90% amino acid, 92% cDNA) to the Alt a 1 subunit from *Alternaria alternata* was identified. Virtual northern blots demonstrated that this homolog, designated Alt b 1 precursor, was highly up-regulated during the infection process of *A. brassicicola* on Arabidopsis. The full length cDNA sequence of Alt b 1 was 815 bp, with an open reading frame of 477 bp. To functionally analyze the role of this allergenic protein, a 500 bp internal cDNA fragment was sub-cloned into the fungal transformation vector pCB1636. PEG-mediated protoplast transformation was performed to create Alt b 1 knockout mutants. We are currently analyzing phenotypic changes in the mutants with disrupted Alt b 1 ORFs.

315. Identification of *Alternaria brassicicola* genes differentially expressed during pathogenesis on *Arabidopsis thaliana* using Suppression Subtractive Hybridization. Robert A. Cramer and Christopher B. Lawrence. Colorado State University, Department of Bioagricultural Sciences Fort Collins, Colorado 80523-1170

Necrotrophic fungal pathogens are responsible for some of the world's most devastating plant diseases. *Alternaria brassicicola* (Schwein.) Wiltshire is a necrotrophic fungus that causes black spot disease on a wide range of cruciferous hosts including the model plant Arabidopsis. The objective of this study was to identify genes up-regulated during the early stages of A. brassicicola infection on Arabidopsis. Suppression subtractive hybridization (SSH) was employed to create a cDNA library enriched for such genes. Fungal spores were germinated either in sterile water or on leaves of the susceptible Arabidopsis ecotype Landsberg erecta (Ler). After a 24 hr incubation period at 24°C, RNA was extracted from these two fungal spore samples and used to create cDNA populations for use in SSH. Subtraction was performed between these cDNA populations to create a library enriched for genes unique to the spores germinated on the plant leaf surface. Up-regulation of clones corresponding to individual genes was confirmed using a dot blot technique coupled with virtual northern analysis. Fifty up-regulated clones were selected and sequenced. Database homology searches using blastn and blastx revealed sequences with homology to a putative arsenite ATPase translocase (ABC transporter), translation initiation factor, various glycoproteins, Alternaria allergen precursor, cyanide hydratase, and formate dehydrogenase that may be involved in pathogenesis. Based on the results obtained, SSH is an effective technique to identify fungal genes that may be important in the early stage of infection. Future research will involve functional characterization of these putatively important pathogenesis-related genes.

316. Molecular Dissection of the *Stagonospora nodorum* - wheat interaction. Peter S. Solomon, Kar-Chun Tan, T.J. Greer Wilson, Robert C. Lee, Simon Ip Cho, Kerrie Parker & Richard P. Oliver. Australian Centre for Necrotrophic Fungal Pathogens, W.A. State Agricultural Biotechnology Centre, Division of Science and Engineering, Murdoch University, Perth 6150, Western Australia, Australia.

The Australian Centre for Necrotrophic Fungal Pathogens (ACNFP) has been recently established on the west coast of Australia. The ACNFP was created to develop an understanding of necrotrophic fungal pathogen/host interactions at the molecular level, particularly those affecting Australian crops. One such project within the centre is focused on the interaction between the fungus *Stagonospora nodorum* and wheat. *Stagonospora nodorum* is the causal agent of

leaf and glume blotch on wheat and is responsible for $$60M (AUD) of crop loss in Australia each year. Whilst also appearing to be an economically important pathogen throughout the world, very little is known at a molecular level about how the fungus infects wheat. We have begun dissecting this interaction using a variety of molecular techniques including the generation of EST libraries, gene expression analysis, bioinformatics and high throughput gene knockouts. Several genes, including those involved in transport, signal transduction and novel metabolic processes, have been characterised by gene disruption and expression analysis. This poster will review the phenotypic effects of these gene disruptions as well as examining their requirement for pathogenicity.

317. Random Insertional Mutagenesis of the fungus *Leptosphaeria maculans*, identifies two pathogenicity genes and leads to discovery of Repeat Induced Point (RIP). Alexander Idnurm *, Leanne M Wilson and Barbara J Howlett. School of Botany, The University of Melbourne, Victoria 3010, Australia. * Current address: Department of Molecular Genetics & Microbiology, Duke University Medical Center, Durham, NC 27110 USA

Insertional mutagenesis generated pathogenicity mutants of *Leptosphaeria maculans*, the dothideomycete that causes blackleg disease of *Brassica napus*. Two mutants had single copy insertions of the plasmid pUCATPH that encodes hygromycin resistance. In one, the isocitrate lyase gene was mutated. As expected, this mutant did not grow on fatty acids including monolaurate and when the wildtype gene was reintroduced, growth on monolaurate was restored and pathogenicity was partially restored. When 2.5 % glucose was added to this mutant, pathogenicity was restored. These findings suggest that the glyoxylate pathway is essential for disease development by *L.maculans*. The second pathogenicity mutant had pUCATPH inserted upstream of an open reading frame of 529 amino acids with a weak database match to*het-s* of *Podospora anserina*. Reintroduction of a wildtype copy of the gene restored this mutant's ability to form lesions on *B. napus* cotyledons. The role of this gene in plant disease is unknown. When seven pathogenicity mutants with multiple insertions of pUCATPH were crossed to an isolate that attacks *B. napus*, progeny with the hygromycin resistance gene were hygromycin-sensitive. Sequence analysis of an amplified fragment of pUCATPH in six clones derived from one ‘silenced' progeny showed mutation of GC to AT on one DNA strand, reminiscent of repeat-induced point mutation (RIP) in *Neurospora crassa*.

318. A putative secondary metabolite cluster of the blackleg fungus, *Leptosphaeria maculans*. Donald M. Gardiner[1] and Barbara J. Howlett[1].[1]School of Botany, Melbourne University, Victoria, 3010 Australia.

The blackleg fungus, *Leptosphaeria maculans* causes major yield losses to canola (*Brassica napus*) worldwide. Random sequencing of clones in a library of Expressed Sequence Tags prepared from mycelia of *L. maculans* grown in complete medium identified a gene with homology to prenyl transferases in the ergot alkaloid biosynthesis cluster of *Claviceps purpurea*, and paxilline biosynthesis cluster of *Penicillium paxilli*. Sequencing of a *L. maculans* cosmid clone containing the prenyl transferase and an overlapping cosmid revealed the presence of a putative cluster of genes with predicted roles in various aspects of secondary metabolism. These genes include a zinc finger transcriptional protein, a peptide synthetase gene, two oxidoreductases, a methyl transferase, two cytochrome P450s, an ATP-binding cassette-type transporter protein and a glutathione-S-transferase. The function of genes in this cluster is being characterised by reverse genetics (gene knockout), transcriptional profiling and by complementation of isolates of a closely related *Leptosphaeria* species lacking this cluster.

319. Regulation of maize-induced genes in *Ustilago maydis*. Jan W. Farfsing, Regine Kahmann and Christoph W. Basse. Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Straße, 35043 Marburg, Germany

The facultative biotrophic fungus *Ustilago maydis* causes smut disease in its host plant maize. We have identified the fungal *mig1* and *mig2* genes as being specifically and strongly up-regulated during biotrophic growth. The *mig2* genes are comprised in a gene cluster of five highly homologous and similarly regulated genes designated *mig2-1* to *mig2-5*. All *mig* genes lack significant homologies in the database. The *mig 1* as well as the *mig2-5* promoters were subject to repression, which was partially relieved in *U. maydis* mutants of *hda1*, encoding the chromatin modifying enzyme histone deacetylase 1. However highest transcript levels were always encountered during biotrophic growth, indicating the presence of additional regulators. Reconstitution experiments comprising *mig2-5* promoter fragments led to the identification of short, positive *cis*-acting elements required for high transcript levels during biotrophic growth. Negative as well as positive *cis*-acting regions of the *mig2-5* promoter were mapped in close proximity to each other within the region from position –240 to –119 upstream of the translational start ATG. All *mig2* promoters

shared significant homologies in this region. For this reason we used this element to screen for the respective regulator based on a yeast one-hybrid assay comprising cDNA libraries from different stages of the fungal life cycle.

320. Prf1 integrates pheromone and cAMP signaling in the phytopathogen *Ustilago maydis*. Florian Kaffarnik, Marc Leibundgut, Philip Müller, Regine Kahmann and Michael Feldbrügge, Max-Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Marburg, Germany

The basidiomycete *Ustilago maydis* causes smut disease on corn. The fungus switches from a haploid form that proliferates by budding to a filamentously growing dikaryon, the infectious form. A complex signalling network in which an evolutionarily conserved cAMP signalling pathway communicates with a MAP kinase branch regulates this developmental programme. To investigate the involved signalling processes we focus on initial events: the fusion of two haploid cells. Like in comparable model organisms such as *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* mating of two compatible cells is controlled by a pheromone/receptor system that regulates conjugation tube formation and gene expression via a MAP kinase module. Here, we present evidence that the activity of pheromone response factor 1 (Prf1), a transcription factor that activates expression of mating type genes, is regulated on the posttranscriptional level by cAMP as well as MAP kinase signalling. This regulation is most likely achieved by the action of the cAMP-regulated protein kinase A and / or the pheromone-activated MAP kinase. Thus, according to our current model Prf1 functions as an integrator between cAMP and pheromone signalling.

321. An ELAV-like RNA-binding protein regulates filament development and pathogenicity in *Ustilago maydis*. Philip Becht and Michael Feldbrügge, Max-Planck Institute for Terrestrial Microbiology, Department of Organismic Interactions, Marburg, Germany

The basidiomycete *Ustilago maydis* causes smut disease on corn. Prerequisite for pathogenic development is the fusion of two haploid cells resulting in formation of a filamentously growing dikaryon, the infectious form. The fusion process is regulated by a tetrapolar mating system that consists of the biallelic *a* locus and the multiallelic*b* locus. The genes at the *a* locus encode lipopeptide pheromone precursors and cognate receptors involved in intercellular recognition. At the *b*locus a pair of homeodomain proteins is encoded that is only functional as a heterodimeric transcription factor with monomers from different allelic origin. Since RNA-binding proteins are key regulators of differentiation processes in other model systems, such as *D. melanogaster* or *C. elegans*, we hypothesise that RNA-binding proteins are among the effectors regulating pathogenic development in *U. maydis*. Here we introduce Rrm4 identified by searching the genomic sequence for RRM domains. Rrm4 contains a novel domain structure containing 3 RRM domains as well as a C terminal polyA domain. Thereby it resembles ELAV proteins as well as polyA-binding proteins. In mating experiments with compatible *rrm4delta* strains a reduced filament formation was observed, indicating that formation of the infectious form is impaired. In plant infection experiments *rrm4delta* strains exhibit a drastically reduced virulence if compared to wild type. Hence, Rrm4 appears to be important for filament development and pathogenicity.

322. An extracellular protease inhibitor from *Phytophthora infestans* targets tomato serine proteases: a counter-defense mechanism? Miaoying Tian, Sophien Kamoun. Department of Plant Pathology, The Ohio State University, Wooster, OH44691, USA

The plant intercellular space is the battlefront where complex interactions between extracellular proteins from the oomycete pathogen *Phytophthora infestans* and its host plants occur. We used data mining of Phytophthora sequence databases to identify eleven genes encoding putative extracellular protease inhibitors (EPIs) with one to three predicted domains of Kazal-type serine protease inhibitors that are commonly found in animals. The EPI proteins did not show similarity to protease inhibitors from plant pathogens or plants suggesting that they might reveal novel molecular mechanisms in plant-microbe interactions. In vitro protease inhibition assays of three purified EPI fusion proteins against several commercial serine proteases revealed that EPI1 highly inhibits bacterial subtilisin, thus confirming the protease inhibition function predicted by bioinformatics. EPI1 was further demonstrated to inhibit and interact with tomato P69 subtilisin-like proteases, among which P69B and P69C are known to be pathogenesis-related proteins involved in plant defenses. We also found that EPI1, but not EPI2 and EPI3, is resistant to degradation by tomato intercellular fluids. Interestingly, EPI1 was able to protect EPI2 from degradation suggesting that inhibition of host proteases might help secreted proteins of Phytophthora resist

proteolytic cleavage in the plant apoplast. Overall our results suggest that inhibition of plant proteases by *P. infestans* EPI1 could form a novel type of defense-counterdefense cross talk between plants and microbial pathogens.

323. cAMP signal transduction regulates virulence of the human-pathogenic fungus *Aspergillus fumigatus*. Burghard Liebmann, Stephanie Gattung, Bernhard Jahn and Axel Brakhage. Microbiology Institute, University of Hannover, Germany and University Hospital, Weisbaden.

Aspergillus fumigatus is an important pathogen of the immunocompromised host, causing pneumonia and invasive disseminated disease with high mortality rates. We have cloned three conserved components of the cAMP signal transduction cascade, the adenylate cyclase (acyA), a Ga subunit (gpaB) and a catalytic subunit of protein kinase A (pkaC). Deletion of the genes revealed, that all components influence growth, development and sporulation of A. fumigatus. Conidia of acyA and gpaB deletion mutants were killed more efficiently in a macrophage assay. Furthermore, a possible cAMP-dependent regulation of the A. fumigatus virulence determinant pksP, which encodes a polyketide synthase, was investigated by constructing a pksP-promoter lacZ-fusion. pksP-expression was significantly reduced in gpaB deletion backround compared with the expression of the gene fusion in a wild-type strain. We also started to investigate the cAMP-dependent pksP-regulation by promoter mutation analysis, in order to fully understand the role of PKSP during infection.

324. The analysis of the phylogenetic distribution of the pea pathogenicity (*PEP*) gene cluster of *Nectria haematococca* MPVI supports the hypothesis of its acquisition by horizontal transfer and uncovers a new fungal pathogen of garden pea: *Neocosmospora boniensis*. Esteban D. Temporini and Hans D. VanEtten. Department of Plant Pathology, University of Arizona, Tucson AZ.

The filamentous fungus *Nectria haematococca* Mating Population VI (MPVI) contains a cluster of genes required to cause disease on pea. The pea pathogenicity or*PEP* cluster is located on a supernumerary chromosome that is dispensable for normal growth in culture. A comparison of the G+C content and codon usage of the genes in the*PEP* cluster indicates that they differ from genes located on non-dispensable chromosomes in this fungus. These features, and the presence of several sequences with homology to transposable elements in this region, suggest the possibility that the *PEP*cluster was acquired through a horizontal gene transfer event. In this work, we show that homologs of the *PEP* genes have a scattered distribution among fungi belonging to the*Fusarium solani* species complex, which are phylogenetically closely related to *N. haematococca* MPVI. However, homologs to most of the *PEP* genes were detected in *Fusarium oxysporum* f. sp. *pisi*, a pea pathogen distantly related to *N. haematococca* MPVI. This phylogenetic incongruence supports the hypothesis of a horizontal transfer origin of the *PEP* cluster. Our analysis has also determined that homologs for all the *PEP* genes are present in*Neocosmospora boniensis*, another member of the *F. solani* complex. A molecular characterization of the *PEP* homologs in this fungus has shown that they are organized as a cluster, which has a different physical organization compared to the *N. haematococca PEP* cluster. In addition, pathogenicity tests have revealed that *N. boniensis* is virulent on pea although no reports have been found to show this species as a naturally occurring pea pathogen.

325. Functions of *Mg-NCS1* and *LPL1*, genes expressed in germ tube of *Magnaporthe grisea*. Takashi Kamakura[1], Ken-ichiro Saitoh[1, 2], Fumi Ishii[2], Masaki Kanamori[2], Minoru Yoshida[1] and Tohru Teraoka[2]. [1]RIKEN Institute, Wako, Japan.[2]Tokyo University of Agriculture and Technology, Fuchu, Japan

The conidial germ tube of the rice blast fungus, *Magnaporthe grisea*, differentiates an infection specialized cell, an appressorium, required for penetration into the host plant. We have constructed a cDNA subtractive differential library from the appressorium forming germ tube. From the library, some genes were pulled out and analyzed. A gene, *Mg-NCS1*, homologous to the Neuronal Calcium Sensor 1 (NCS1) family, was isolated and null mutants were generated in order to see its function. Different from other organisms' NCS1 family,*Mg-NCS1* seemed to play an important role in tolerance against pH stress. Another gene,*LPL1*, was homologous with lysophospholipase gene. The disruptant of *LPL1*reduced ability to differentiate appressorium on hydrophobic surface and also showed delay of formation of infection-peg on onion epidermis strip and rice leaf sheath compared with the wild-type strain. Since phospholipids metabolic pathways are related to glycerol and diacylglycerol biosynthesis, *LPL1* may be involved in the penetration of infection peg. However, these genes did not seem to have essential functions for pathogenic cycle because both of null mutants (Mg-ncs1- and Lpl1-) kept potential ability to make lesions in rice leaves.

326. The Cross-Pathway Control of the Opportunistic Pathogen *Aspergillus fumigatus* Cloning and Characterisation of its Transcriptional Activator CPCA. Sven Krappmann[1], Utz Reichard[2], Verena Grosse[1], and Gerhard H. Braus[11]Institute of Microbiology and Genetics, Department of Molecular Microbiology and Genetics. [2]University Hospital, Center for Hygiene and Human Genetics, Department of Bacteriology. Georg-August University, Goettingen, Germany

The filamentous saprophyte *Aspergillus fumigatus* accounts for the majority of aspergillosis incidents, which represent a severe threat mainly to immunocompromised individuals. Detailed knowledge on factors contributing to pathogenicity of this opportunistic pathogen is scarce with only few determinants identified. Nutritional requirements and metabolic features rendering the fungus infective have not been investigated in great detail. We have focused on the Cross-Pathway Control (cpc) system of *A. fumigatus*, a global regulatory network acting on amino acid biosynthesis to counteract conditions of starvation or imbalance. The cpcA locus encoding the transcriptional activator protein of the system was identified and characterised. Its gene product represents a highly conserved protein that is the functional orthologue of Gcn4p, the yeast master regulator of gene expression upon starvtion and stress. Generation of *cpcA* deletion mutants revealed its central role for the cpc response of this fungus upon amino acid starvation. Next steps aim at the impact of the cpc system on pathogenicity of *A. fumigatus*, the current status of the project will be presented.

327. Molecular characterization of a calcineurin A gene, *MgCNA*, in *Magnaporthe grisea*. Jinhee Choi, Yangseon Kim, Soonok Kim and Yong-Hwan Lee, School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

Magnaporthe grisea, the causal agent of the rice blast, differentiates a specialized infection structure called an appressorium that is crucial for host plant penetration. Pharmacological data suggests that calcium/calmodulin-dependent signaling is involved in appressorium formation in this fungus. Calcineurin is a calcium/calmodulin-activated protein phosphatase composed of a heterodimer of a catalytic (CnA) and a regulatory (CnB) subunit. To understand the role of calcineurin on appressorium formation at molecular level, a gene (*MgCNA*) encoding calcineurin catalytic subunit was cloned and characterized from *M. grisea* 70-15. *MgCNA* contains an ORF of 1,920 bp, encoding 537 amino acids. The overall order and amino acids of protein domains of MgCNA are conserved with those of other filamentous fungi. Transformants expressing antisense of *MgCNA* exhibited significant reductions on mycelial growth rate, conidiation and appressorium formation, and different colony morphology and hyphal branching patterns. These pleiotropic effects suggest that calcineurin A plays important roles on signaling transduction pathways involved in fungal developments of *M. grisea*.

328. Ruderal and combatative strategies in insect pathogenic fungi examined by EST analysis. Gang Hu, Florian M. Freimoser and Raymond J. St. Leger. Department of Entomology, University of Maryland, 4112 Plant Sciences Building, College Park, MD, 20742, USA

Metarhizium anisopliae (Ascomycota) and *Conidiobolus coronatus* (Zygomycota) are facultative saprophytes that are pathogenic to many insect species. About 2000 EST cDNA clones from each species were sequenced to analyze gene expression during growth on host insect cuticle and/or nutrient rich media. Proteases were the commonest transcripts with both species producing multiple subtilisins, as well as trypsin, metalloprotease and aspartyl protease activities capable of degrading host tissues and disabling anti-microbial peptides. However, compared to *M. anisopliae*, *C. coronatus* produced fewer extracellular hydrolases (e.g., no phospholipases), antimicrobial agents, toxic secondary metabolites and no ESTs with putative roles in the generation of antibiotics. Instead, *C. coronatus* produced a much higher proportion of ESTs encoding ribosomal proteins and enzymes of intermediate metabolism consistent with the rapid growth characteristics of *C. coronatus*. These results are consistent with *M. anisopliae* using a combatative strategy to defend captured resources, while *C. coronatus* has modified the saprophytic ruderal-selected strategy using rapid growth to overwhelm the host and exploit the cadaver before competitors overrun it. Both strategies are consistent with specialization to pathogenicity. Thus, induction of proteases by host cuticles (mucoralean fungi do not produce proteases under these conditions) demonstrates that *C. coronatus* is adapted to entomopathogenicity.

329. EST and microarray analysis of pathogenicity factors of the insect pathogen *Metarhizium anisopliae*.
Gang Hu, Florian M. Freimoser, Steven Screen, Savita Bagga and Raymond St. Leger Department of Entomology, University of Maryland, 4112 Plant Sciences Building, College Park, MD, 20742, USA

ESTs and microarrays offer unprecedented opportunities for analysis of pathogenicity in fungi. ESTs of the insect pathogen *Metarhizium anisopliae* were obtained under growth conditions that optimize the secretion of many known pathogenicity factors. The EST sequences revealed new unsuspected stratagems of entomopathogenicity and previously unreported categories of biologically active molecules, as well as many enzymes involved in the degradation of host barriers. For example, *M. anisopliae* produces more proteinolytic enzymes than any other fungus studied to date. These comprise at least 11 different subtilisins as well as multiple trypsins, metalloproteases, chymotrypsins, etc. To analyze gene expression during the infection process cDNA fragments were arrayed onto glass slides. Genes encoding components of membrane biogenesis, synthesis of cell wall components, storage or mobilization of nutrient reserves and protein folding (particularly calnexin-always associated with protein secretion in 2575) are also highly expressed during appressorial differentiation indicative of manufacture and "remodeling" of cell structure. Other features of *M. anisopliae* physiology highlighted by this work include the production of antimicrobial molecules (presumably to defend limited resources within the insect cadaver) and the very early cuticle-induced production of variety of transporters and permeases that allow the fungus to "sample" the cuticle by absorbing peptides and amino acids and then respond with secretion of a plethora of proteins. Our results also suggest that many differences in the phenotypes of different strains result from genes with similar sequences but dissimilar expression patterns.

330. Patchy distribution of trypsin genes in fungi.
Gang Hu, Florian M. Freimoser steven Screen, Savita Bagga, and Raymond J. St. Leger Department of Entomology, University of Maryland, 4112 Plant Sciences Building, College Park, MD, 20742, USA

The trypsin family of proteases have homologs in streptomycetes, five pathogenic ascomycetes and animals. This patchy distribution is consistent with: 1) components of the genetic apparatus of ascomycetes being derived from an ancestor of the streptomyces via horizontal gene transfer, or 2) rampant gene duplication, divergence and gene loss in different fungal lineages. In order to reconstruct the evolution of trypsin diversity we related the presence or absence of trypsins to the phylogenetic relationship of 46 representative fungi. Our results are consistent with niche-specific traits, that are traits shared by fungi that occupy the same niche irrespective of phylogenetic position. We found that trypsins are produced by: 1) many plant and insect pathogenic pyrenomycetous ascomycetes; 2) the basidiomycete Septobasidium canescens that has a symbiotic relationship with aphids that occasionally includes digesting them, and 3) some entomopathogenic entomophoralean zygomycetes (Conidiobolus coronatus, Zoophthora spp.). Trypsins are lacking in saprophytic ascomycetes including yeast, N. crassa and A. nidulans as well as saprophytic zygomycetes and basidiomycetes. The observed phylogenetic distribution of the trypsin orthologs is largely in agreement with an unconstrained rDNA tree that is itself consistent with the current consensus on fungal phylogeny. This indicates that these proteins have diverged in parallel with the organisms in which they are expressed. Several species such as Paecilomyces, which have no trypsins, are nested between species that do, indicating that the trypsin has been lost in the lineage leading to that species. Overall, comparative studies suggest that individual genes, such as the trypsins have been lost many times independently in different lineages, and that the flux of genes is an ongoing process. This is clearly seen in the specialized M. anisopliae strain 324 that unlike generalist strains of the same species contains a silent trypsin pseudogene.

331. In *Ustilago maydis* the MAP kinase Kpp6 is required for successful penetration of the plant surface.
Andreas Brachmann, Jan Schirawski, Philip Müller and Regine Kahmann. Max Planck Institute for terrestrial Microbiology, Marburg, Germany.

To complete its life cycle, the plant pathogenic fungus *Ustilago maydis* has to invade the host plant tissue. During this process dikaryotic filaments form appressorium-like infection structures from where growing hyphae extend into the plant tissue. In *Ustilago maydis* filamentous growth and pathogenic development depend on the expression of the regulatory bE/bW complex. We have identified by RNA fingerprinting a b-regulated gene, *kpp6*, encoding a MAP kinase with similarity to other fungal MAP kinases involved in mating and pathogenicity. Kpp6 is unusual in that it contains an N-terminal domain unrelated to other proteins. *kpp6* deletion mutants are unaffected in mating and dikaryon formation but show attenuated pathogenic development. $kpp6^{T355A,Y357F}$ mutants carrying a non-activatable

allele of *kpp6* are severely compromised in pathogenicity. These strains can still form appressoria, but are defective in the subsequent plant surface penetration step. A Kpp6-GFP fusion is expressed during all stages of the sexual life cycle. Expression of the *kpp6* gene yields two transcripts that are differentially expressed. We have analyzed these transcripts and present evidence that the smaller one is regulated through Prf1 while the longer one is regulated indirectly through the bE/bW heterodimer.

332. Signalling in the plant pathogen *Ustilago maydis*: A comparative genomics approach. Heiko Eichhorn, Joerg Kaemper, Philip Mueller and Regine Kahmann. Max Planck Institute for terrestrial Microbiology, 35043 Marburg, Germany.

Ustilago maydis is the causative agent of corn smut disease. The pathogenic dikaryotic form is generated after mating of two compatible cells. Results from several laboratories have shown that the signalling pathways required for transmission of the pheromone signal during mating are also needed during pathogenic development. In particular, the components of a MAP kinase module as well as tightly regulated cAMP signalling are needed for disease progression. Recent results suggest that these pathways have partially overlapping as well as distinct functions. To analyse these pathways in more detail we have performed a comparative transcriptome analysis. To this end, strains were generated which allow to activate the different signalling pathways. Wild type alleles or non-phosphorylatable alleles of the two MAP kinases *kpp2* and *kpp6*, respectively, were introduced into strains that harbour a constitutively active allele of the MAPKK *fuz7*. The expression of the *fuz7* allele is made inducible by the *crg1* promoter which is ON in arabinose and OFF in glucose. In addition we have generated a strain that expresses the catalytic subunit of the PKA (*adr1*) under control of the *crg1* promoter. The transcription profile of pheromone stimulated wild type cells was included for comparison. RNA was isolated at various time points and analysed by whole genome microarrays (Affymetrix). We will present these data and discuss their impact for the identification of genes that are functionally significant for pathogenic development.

333. Establishment of the sexual cycle of *Cryptococcus neoformans* variety*grubii* and virulence of congenic a and alpha isolates. Kirsten Nielsen and Joseph Heitman. Dept. Molecular Genetics and Microbiology, Duke University Medical Center and The Howard Hughes Medical Institute, Durham, NC

Cryptococcus neoformans is an opportunistic human pathogen that infects the central nervous system of immunocompromised individuals. This basidiomycete has evolved into three distinct varieties. A heterothallic sexual cycle with haploid **a** and alpha cells has been defined for one variety (*neoformans*, serotype D), but the most common pathogenic variety (*grubii*, serotype A) was thought to be clonal and restricted to the alpha mating type. We have recently identified multiple serotype A **a**-mating type strains from a worldwide screen of greater than 500 strains. We characterized one of these unusual isolates (strain 125.91) and show it is an authentic haploid serotype A **a**-mating type strain. The **a**A strain 125.91 is capable of mating with a subset of pathogenic serotype A alpha strains to produce filamentous dikaryons with fused clamp connections, basidia, and viable recombinant basidiospores. Congenic serotype A **a** and alpha strains were generated and analyzed for virulence potential in animal models. These strains provide the platform to conduct a broad scale genetic analysis of the molecular determinants of virulence.

334. *Ustilago maydis uac* mutants elicit a host response in maize leaves. Aarthi Gopinathan, Karen Snetselaar, Michael McCann. Biology Department, Saint Joseph's University, Philadelphia PA

The basidiomycete *Ustilago maydis* causes corn smut disease. This dimorphic fungus is normally non-pathogenic when growing vegetatively by budding. Infection occurs when compatible cells form mating filaments that fuse, and the resulting filamentous dikaryon enters host tissues. Adenylate cyclase (*uac*) mutants of the fungus are constitutively filamentous (Gold S, Duncan G, Barrett K, Kronstad J; 1994; Genes and Dev 8:2805-16), yet they are non-pathogenic. Filaments formed by *uac* mutants differ from mating and infection filaments in features of polarity and cell division. Exogenous cAMP restores the ability of the *uac* mutants to produce mating and infection filaments in vitro and on host surfaces. However, although *uac* mutants provided with cAMP make infection filaments that grow in plant tissue and may initiate tumor formation, the tumors fail to complete development. To determine whether host defense responses accompanied the impaired pathogenicity, we used diaminobenzidine to localize H_2O_2 in the presence of peroxidase, indicating a hypersensitive response, (Vanacker, H, Carver, T, Foyer, C; 2000; Plant Phys 123:1289-1300). Leaves inoculated with *uac* mutants supplemented with exogenous cAMP produced a

much stronger host response than leaves inoculated with wild-type cells where no cAMP was added. However, control inoculations involving wild-type cells and added cAMP also resulted in a host response. These and additional experiments indicate that regulation of cAMP is required for normal completion of the *U. maydis* pathogenic program. The work was partially funded by NSF MCB 9807807 to K. Snetselaar and M. McCann.

335. The HMG-box protein Rop1 is essential for pheromone-responsive gene expression in *Ustilago maydis*. Thomas Brefort, Philip Muller and Regine Kahman. Max-Planck-Institute for terrestrial Microbiology, 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 so-called pheromone response leading to an activation of the HMG-domain transcription factor Prf1. Prf1 binds to the PRE-boxes located in the promoters of the *a*- and *b*-genes. As a result, stimulated wildtype cells show elevated transcription of these genes as well as conjugation tube formation, while *prf1* mutants do not. Here, we present the identification of *rop1* encoding a second sequence-specific HMG-domain protein. While the HMG-domain of Rop1 is 17,7% identical to the HMG-domain of Prf1, it shows highest identity (42,6%) to the HMG-domain of Pcc1 of *Coprinus cinereus*. *rop1* deletion strains display a severe mating defect and do not form conjugation hyphae upon pheromone stimulation. Northern analyses revealed that *rop1* is essential for *prf1*, *mfa1*, *pra1* as well as *b* gene expression. Since constitutive expression of *prf1* fully complements the observed mating defect of*rop1* deletion strains it is likely that Rop1 regulates *prf1* gene transcription. We are now investigating whether Rop1 binds directly to the *prf1* promoter or whether its effect on *prf1* gene transcription is indirect.

336. Functional analysis of a thiamine biosynthetic gene in the interaction of *Epichloë typhina* with perennial ryegrass. Xiuwen Zhang[1], Michael Christensen[2], Barry Scott[1]. [1]Institute of Molecular BioScience, Massey University, Private Bag 11 222, Palmerston North, New Zealand. [2]AgResearch Limited, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand.

Epichloë/Neotyphodium endophytes are a group of clavicipitaceous fungi that form symbiotic associations with temperate grasses. The asexual *N. lolii* form asymptomatic mutualistic associations with ryegrass whereas the sexual *E. typhina* behaves as a mutualist during the vegetative phase of plant growth but switches to epiphytic growth and formation of an external stroma upon development of the floral inflorescence. We are interested in the metabolic interaction between these endophytes and their perennial ryegrass host. We have chosen to examine the role of endophyte thiamine biosynthesis in this interaction, because of its key role as a coenzyme in primary cellular metabolism. The orthologue (*thi1*) of*Saccharomyces cerevisiae THI4* was isolated from *N. lolii* and *E. typhina* by PCR using degenerate primers designed to conserved regions of known thiazole biosynthetic genes. This gene is strongly expressed in culture and *in planta* and shows alternative splicing, with distinct patterns of the isoforms expressed under different nutritional conditions. A knockout of the *E. typhina thi1* has been constructed and shown to have reduced hyphal density and branching compared to the wild-type on defined media lacking thiamine. Both thiamine and thiazole complemented this defect. No differences in infectivity were observed between wild-type and mutant in their ability to establish stable artificial associations with perennial ryegrass. However, some differences were observed in host colonisation, with the mutant strain behaving more like the asexual *N. lolii*. Both wild-type and mutant formed stromata on reproductive tillers, and, unexpectedly, both formed some stromata on vegetative tissue.

337. Smu1, a Ste20p homologue from *Ustilago maydis* with roles in mating and pathogenicity. David Smith[1], Zhanyang Yu[1], Scott Gold[2], and Michael H. Perlin[1]. [1] University of Louisville, Louisville, Kentucky, USA and [2] University of Georgia, Athens, Georgia, USA

Ste20p is a member of the PAK family of protein kinases responsible for regulation of a series of mitogen-activated protein kinase (MAPK) signal transduction cascades conserved from yeast to humans. We isolated genes, *smu1* and *smtE*, encoding homologues of the PAK-like Ste20p from, respectively, the corn smut, *U. maydis*, and from the anther smut, *Microbotryum violaceum*. Though both proteins were similar to the PAK-like kinases, they were distinct from the germinal-center kinases, including Don3, also from *U. maydis*. When both *U. maydis* partners in a mating pair were disrupted for *smu1* there was significant impairment of mating. Over-expression of the catalytic

region of the *M. violaceum* SmtE failed to complement *U. maydis* with *smu1* knock-outs. Interestingly, such expression interfered with wildtype mating and made the mating defect of the *smu1* knock-out even more pronounced. *Smu1* knock-out strains also showed significantly reduced ability to cause disease when used to infect maize. In such infections, the majority of symptoms were limited to those observed early in infection; gall formation and plant death were almost never observed. Thus, unlike the Don3 kinase involved in cell separation, *smu1* is part of the mating pathway, and its disruption greatly impairs the ability of *U. maydis* to cause disease.

338. Genome Rearrangement in *Magnaporthe grisea*: Translocation of an Avirulence Gene. M.W. Harding, M.A. Mandel and M.J. Orbach. University of Arizona, Department of Plant Pathology, Tucson, AZ.

We are working to characterize the fungal effector (avirulence) gene *AVR1-MARA* from *M. grisea*. An incompatible (avirulent) phenotype is observed on the rice cultivar Maratelli challenged with strain 4224-7-8. Incompatibility is controlled by a single gene (*AVR1-MARA*) that does not readily or spontaneously mutate to virulence.*AVR1-MARA* originated in G-22, an *M. grisea* strain that is pathogenic on finger millet. G-22 is highly fertile and was backcrossed to a rice pathogen (0-17) to increase fertility in a rice pathogen field isolate (Dobinson & Hamer, 1992). Incompatibility of 4224-7-8 progenitor strains on Maratelli is also defined by a single genetic locus. However, we discovered that incompatibility in progenitors segregated independently with respect to *AVR1-MARA*when crossed with 4224-7-8. Our working hypothesis is that these two unlinked incompatibility loci contain the same effector gene. Data that support this hypothesis will be presented. We propose that during a backcross with G-22 (cross 4134) an insertional translocation was responsible for moving the effector gene from the ancestral location in G-22 to the*AVR1-MARA* locus described in 4224-7-8 (Mandel et al., 1997). DNA sequence from G-22 and 4224-7-8 indicate that a region of AT-rich sequence larger than 40kb moved during the translocation. Mandel et al. 1997. MPMI 10:1102-1105. Dobinson & Hamer. 1992. *Magnaporthe grisea*. In: Molecular Biology of Filamentous Fungi. U. Stahl & P. Tudzynski, eds. Weinheim, NY.

339. The role of reactive oxygen species in plant-pathogen interaction. P. Tudzynski, K. B. Tenberge, S. Joshi, S. Moore, Y. Rollke, E. Nathues; Institut fuer Botanik, Westf. Wilhelms-Universitaet, Schlossgarten 3, D-48149 Muenster, Germany

One of the earliest reactions of plants against pathogens is the transient formation of reactive oxygen species (ROS), termed in analogy to mammalian systems "oxidative burst". Its impact on plant defense has been studied in detail; the active and passive role of the pathogen facing this oxidative stress during the early stages of interaction is less clear. Two different strategies seem to exist: in the necrotroph Botrytis cinerea the formation of ROS in planta is directly correlated with aggressiveness of fungal isolates ; there is evidence for production of ROS by the fungus itself, triggering enhanced production by the plant. This leads to rapid death of plant tissue, facilitating fungal growth. In more balanced systems like in the biotrophic cereal pathogen *Claviceps purpurea*, the fungus obviously tries to overcome the oxidative stress by building up a protective shield of secreted AOS-scavenging enzymes. We are studying both types of interactions using cytological, biochemical and molecular genetic techniques including functional analysis by targeted gene inactivation. We will focus here on the *C. purpurea*/rye system and present evidence that the oxidative stress response system has impact on the interaction. We have identified a bZIP transcription factor (cptf1) involved in oxidative stress response, which controls catalase activity and is essential for full virulence of the fungus on rye.

340. Isolation and characterization of genes preferentially expressed during asexual sporulation in the oomycete plant pathogen *Phytophthora cinnamomi.* Reena Narayan, Weixing Shan, and Adrienne R. Hardham. Plant Cell Biology Group, Research School of Biological Sciences, Australian National University, Canberra, Australia.

The genus Phytophthora contains at least 60 species, many of which are destructive pathogens causing diseases in hundreds of commercially important plants. *P. cinnamomi* is capable of infecting over a thousand plant species and causes severe economic and ecological losses to agriculture and forest industries in Australia and worldwide. Under nutrient-deprived conditions, vegetative hyphae of *P. cinnamomi* sporulate to produce multinucleate, asexual sporangia that cleave to form uninucleate, motile zoospores, which are the primary means of infection of new hosts. Our current understanding of the molecular basis of sporulation is extremely limited. Identification and characterization of *Phytophthora* genes that serve key roles in sporulation and spore function would make a

significant contribution towards increasing our understanding of these processes. In this study, differential hybridization techniques were used to screen over 5000 cDNA clones from a *P. cinnamomi* cDNA library made from an early stage of sporulation, and 328 putative sporulation-specific genes were isolated and partially sequenced. Candidate genes were identified through Genbank and *Phytophthora* Genome Consortium database comparisons. Of the 328 sequenced clones, 195 were found to represent unique genes of which 27% were homologous to metabolic and structural proteins, 31% were homologous to ribosomal proteins, and 42% were unknown genes. Three candidate genes were selected for further molecular characterization. A macroarray of the unique genes was screened with probes made from mRNA isolated at nine different stages in the sporulation process and cohorts of genes expressed at different stages of sporulation were identified. The macroarray results were complemented by RNA blot analysis.

341. Identification of a cysteine-rich protein secreted by *Fusarium oxysporum* during growth in tomato xylem vessels. Martijn Rep[1], Charlotte van der Does[1], Michiel Meijer[1], Henk L. Dekker[2], Petra M. Houterman[1], Chris G. de Koster[2] and Ben J.C. Cornelissen[1]. [1]Plant pathology and [2]Mass spectrometry, Swammerdam Institute for Life Sciences, University of Amsterdam

Fusarium oxysporum f. sp. *lycopersici* colonizes tomato plants through invasion of xylem vessels, resulting in wilt disease. Proteins secreted by the fungus in xylem sap are likely to play a crucial role during colonization. On the one hand, they can serve to promote colonization, for instance through suppression of plant defense mechanisms. On the other hand, they may elicit plant defense responses. In order to clarify the molecular basis of *Fusarium* pathogenicity, we set out to identify proteins secreted by the fungus in tomato xylem vessels. Besides several tomato PR proteins, a 12 kD *Fusarium* protein (SIX1, for Secreted in Xylem 1) has now been identified. The corresponding gene was isolated and potentially encodes a 30 kD protein, from which SIX1 is derived through proteolytic processing. The protein is cysteine-rich and does not resemble any other known protein. In a mutant with an altered virulence phenotype (less virulent on some tomato cultivars, more virulent on another), the *SIX1* gene is lost along with neighbouring sequences. Characterization of the genomic locus of the *SIX1* gene and that of a truncated homolog on the same chromosome revealed the presence of several repetitive elements. The potential role of SIX1 in pathogenicity and/or avirulence is being investigated through complementation and knock-out studies.

342. Signalling and pathogenicity in the grey mould *Botrytis cinerea*. Christian Schulze Gronover, Philipp Hantsch and Bettina Tudzynski. Westfälische Wilhelms-Universität Münster, Institut für Botanik, Schlossgarten 3, 48149 Münster, Germany

Heterotrimeric G proteins were shown to play an important role in pathogenicity of many fungi. We cloned and deleted two different genes (*bcg1* and *bcg2*). *bcg1* mutants differ in colony morphology from the wild-type, show a reduced growth rate and extracellular protease activity. Tomato and bean leaves inoculated with conidia from *bcg1* mutants caused only primary necrosis, but never spreading lesions. *bcg-2* mutants developed secondary lesions but much slower than the wild-type. Biochemical analysis showed that *bcg1*, but not *bcg2* mutants lost the ability to produce the phytotoxin botrydial. In a molecular approach (SSH) we identified *in planta* expressed genes which are not longer expressed in *bcg1* mutants. Furthermore, we deleted the *B. cinerea* adenylate cyclase gene (*bac*) resulting in reduced vegetative growth and a colony morphology reminding that of *bcg1* mutants. Conidia of *bac* mutants were still able to germinate, to penetrate bean leaves, and, in contrast to *bcg1* mutants, to form soft rot. However, the development of secondary spreading lesions is much slower, and in contrast to the wild-type, no conidiation was obtained on bean leaves. In contrast to *bcg1* mutants, *bac* mutants still produce botrydial and extracellular proteases. The much stronger effect of *bcg1* mutation on pathogenicity in comparison to the *bac* mutation suggests that BCG1 controls at least one more signalling pathway in addition to the cAMP pathway. In addition, several protein kinase-encoding genes, e.g. a homologue of the S. cerevisiae sch-9 gene, and genes encoding small GTP-binding proteins of the ras family were cloned and their role in pathogenicity was analyzed.

343. Signalling cascades regulating growth and virulence in *Fusarium oxysporum*. Jesús Delgado, Ana Lilia Martínez-Rocha, Raquel Roldán, Carmen Velasco, M. Isabel G. Roncero, Michael W. Rey and Antonio Di Pietro. Universidad de Cordoba, Department of Genetics, Cordoba, Spain.

Fusarium oxysporum is a soilborne fungus that causes vascular wilt disease in a wide variety of crops and has also been reported as an emerging opportunistic pathogen of humans. A signalling cascade controlled by the

extracellular-regulated mitogen-activated protein kinase (MAPK) Fmk1 was shown previously to be required for infection. Here we report the presence of a second, independent pathway that regulates development and virulence in *F. oxysporum* by identifying one of its components, the G protein beta subunit Fgb1. Strains carrying either a *fgb1* loss-of-function mutation (delta *fgb1*) or a dominant activating allele (*fgb1*^{W115G}) show strongly reduced virulence on tomato. Similar to delta *fmk1* strains, *fgb1* mutants produce altered levels of extracellular virulence factors such as polygalacturonases and hyphal hydrophobicity determinants. Levels of Fmk1 phosphorylation in *fgb1* mutants are comparable to those in the wild type strain as shown by immunoblot analysis with anti-phospho-p44/p42 MAPK antibodies, suggesting that Fgb1 does not signal upstream of Fmk1. Delta *fgb1* mutants display a strongly elongated and unbranched hyphal growth pattern which is reversed by the protein kinase A (PKA) inhibitor H-89. Conversely, strains overexpressing the dominant activating *fgb1*^{W115G} allele show increased branching and premature submerged conidiation, which can be reversed by the phosphodiesterase inhibitor IBMX. We propose that the G-protein beta subunit Fgb1 controls proliferative growth, differentiation and virulence in *F. oxysporum* by negatively regulating a cAMP-dependent PKA cascade.

344. *ACE1*: a complex evolution from avirulence to virulence in populations of the rice blast fungus *Magnaporthe grisea*. Isabelle Fudal[1], Heidi U. Böhnert[1], Didier Tharreau[2], Jean-Loup Notteghem[3] and Marc-Henri Lebrun[1]. [1]CNRS/Bayer, France. [2]CIRAD-CA, France.[3]ENSA-M, France.

Rice resistance to the blast fungus *Magnaporthe grisea* depends on specific interactions controlled by fungal avirulence genes and their corresponding plant resistance genes. The avirulence gene *ACE1* that interacts with the rice resistance gene *Pi33* was isolated by positional cloning and encodes a combined polyketide synthase (PKS)/nonribosomal peptide synthetase (NRPS) (4035 aa). The basis of virulence towards *Pi33* in nature was investigated through population studies. Most rice-pathogenic *M. grisea* isolates collected at diverse locations such as China, Philippines or Columbia turned out to be avirulent towards*Pi33*. The rare virulent isolates were detected in different geographic populations but are genetically related. We analysed *ACE1* polymorphism in a subset of avirulent and virulent isolates representative of the world-wide diversity. This revealed that all avirulent isolates have an *ACE1* allele similar to the avirulent isolate Guy11, while virulent isolates display significant polymorphism. Three distinct virulent alleles can be distinguished. The first type of virulent isolate has a RFLP pattern identical to Guy11-*ACE1* suggesting that it may result from point mutations in *ACE1* open reading frame (ORF) or promoter. CM28-*ACE1* allele differs from the Guy11-*ACE1* allele by several restriction site polymorphisms. Sequencing revealed 12% nucleotide diversity when compared to Guy11-*ACE1* suggesting that the CM28-*ACE1* allele is not derived from the Guy11-*ACE1* allele by recent accumulation of point mutations. The third virulent*ACE1* allele (PH14-*ACE1*) has a combination of patterns corresponding to Guy11-*ACE1* and CM28-*ACE1* patterns. The corresponding alleles are localised on two different chromosomes, indicating that these normally haploid isolates are partially diploid for *ACE1*. A possible scenario of *ACE1* evolution in *M. grisea* populations will be proposed.

345. A Data Mining Strategy to Identify *in planta* Induced Genes from the Oomycete Pathogen *Phytophthora infestans*. Luis da Cunha, Edgar Huitema, Miaoying Tian and Sophien Kamoun. Department of Plant Pathology, The Ohio State University - OARDC, Wooster, OH

The oomycete *Phytophthora infestans* causes late blight, a devastating disease of tomato and potato. A key step in understanding pathogenicity of *P. infestans* is to define the transcriptional changes that take place during colonization of host tissue. For example, *in planta*-induced (*ipi*) genes are more likely to encode virulence or avirulence factors and form attractive candidates for detailed functional analyses. Here, we describe a strategy to identify *ipi* genes from expressed sequence tags (ESTs) obtained from infected tomato tissue. To select a subset of *ipi* candidate genes, computational analyses based on GC counting and differential BLAST searches against tomato and *Phytophthora* databases were performed on 2808 "interaction" ESTs. A total of 523 ESTs (19%) were predicted to originate from *P. infestans*. Additional BLAST searches against 72.000 in vitro *P. infestans* ESTs identified a set of 55 sequences that are over-represented in the interaction. To validate this data mining strategy, we performed PCR on *P. infestans* and tomato genomic DNA, semi-quantitative RT-PCR, and northern blot analyses. All genes examined were confirmed to be from *P. infestans*. Of these, 52 were expressed during colonization of tomato and 10 were found to be up-regulated during infection using semi-quantitative RT-PCR. Current work focuses on functional analyses of the novel *ipi*genes to determine whether they play a role in pathogenesis.

346. Secondary metabolism and avirulence in *Magnaporthe grisea*: is *ACE1* part of an avirulence gene cluster? Heidi U. Böhnert, Isabelle Fudal, Anne-Elodie Houlle and Marc-Henri Lebrun. UMR 1932 CNRS / Bayer CropScience, Lyon, FRANCE.

Resistance of rice to the blast fungus *Magnaporthe grisea* depends on specific interactions controlled by fungal avirulence genes and their corresponding plant resistance genes. The avirulence gene *ACE1* that interacts with the rice resistance gene *Pi33* encodes a combined polyketide synthase (PKS)/nonribosomal peptide synthetase (NRPS), a novel type of eukaryotic enzyme involved in the biosynthesis of an as yet unidentified secondary metabolite. Comparison of *ACE1* to known fungal PKS shows that it is related to LNKS from *Aspergillus terreus*, involved in the biosynthesis of lovastatin. *ACE1* is expressed exclusively in penetrating appressoria. High turgor of the appressorial cell appears to be required but not sufficient for the induction of *ACE1* expression. Since Ace1p is localized in the cytoplasm of the appressorium and the enzymatic function of Ace1p required for avirulence, we conclude that the signal recognized by resistant rice is not Ace1p itself, but the secondary metabolite produced by Ace1p. Sequence analysis of the genomic region adjacent to *ACE1* revealed a cluster of genes potentially involved in secondary metabolism. Several of these appear to be co-regulated with*ACE1*. Our current research focuses on two aspects: identification of the common regulator of the *ACE1* gene cluster and whether the entire gene cluster is involved in avirulence.

347. Characterisation of Trehalose Biosynthetic and Metabolic Mutants of *Magnaporthe grisea*. Joanna M. Jenkinson and Nicholas J. Talbot. School of Biological Sciences, University of Exeter, Washington Singer Laboratories, Perry Road, Exeter, EX4 4QG, United Kingdom.

The heterothallic ascomycete *Magnaporthe grisea* causes rice blast disease of cultivated rice. The potentially devastating effects of this disease are determined by the ability of the fungus to mechanically penetrate the plants cuticle and epidermis. This force is due to the enormous hydrostatic turgor that accumulates within the appressorium as a result of the high concentrations of glycerol present. Glycogen, lipid and trehalose are thought to be potential precursors for glycerol biosynthesis in *M. grisea.* Trehalose is also one of the major carbohydrates in dormant conidia and can act as a stress metabolite.

The aim of this project is to understand the role of trehalose in the infection cycle of *Magnaporthe grisea*. Using a trehalose-6-phosphate synthase mutant we have shown that trehalose synthesis is required for plant infection and affects the ability of appressoria to generate turgor. We have also identified two trehalases, but only one of these activities is important for pathogenesis, affecting post-penetration development.

348. Structure-function relationship studies on the CBEL glycoprotein of *Phytophthora parasitic*a var. *nicotianae*, by PVX expression in N. benthamiana. E. Gaulin[1,2], T. Torto[2], Y. Martinez[1] M. Khatib[1], A. Bottin[1], M.T. Esquerré-Tugayé[1], S. Kamoun[2], and M. Rickauer[1]. [1]Pôle de Biotechnologie Végétale UMR5546, B.P.17 Auzeville, 31326 Castanet-Tolosan, FRANCE[2] Dept of Plant Pathology, OARDC, 1681 Madison Av., Wooster, Ohio, USA

CBEL is a cell wall glycoprotein produced by *Phytophthora parasitica* var. *nicotianae*, an oomycete pathogen of tobacco. It binds to cellulose and plant cell walls in vitro and induces defense reactions in the host plant (1). The protein structure of CBEL consists of two repeated domains separated by a linker region ; each domain contains a motif similar to the cellulose-binding domain (CBD) found in fungal glycanases. In order to determine the roles of the different domains in elicitor activity of CBEL, we adopted the PVX expression system for our studies. The coding sequence of CBEL, including its proper signal peptide sequence, as well as various deletions and point mutations, were introduced into the pGR106 expression vector (2). Production of CBEL in planta induces necrosis in *N. benthamiana* leaves, leading to death of the whole plant. The necrosis-inducing activity, together with western blot analysis and immunocytolocalisation of CBEL, show that the oomycete secretion signal peptide directs CBEL towards the cell wall in *N. benthamiana*. Results obtained with mutant forms of CBEL indicate that the CBDs are involved in its necrosis-inducing activity, and hence in its perception by the plant cell.

1. Villalba-Mateos et al., 1997. Mol. Plant-Microbe Interact. 10, 1045-1053 2. We thank D. Baulcombe, Norwich, for the gift of pGR106

349. The corn pathogen *Ustilago maydis* responds to triglycerides by switching from budding to filamentous growth. J. Klose, M. Moniz de Sa and J. Kronstad. Biotechnology laboratory, Department of Microbiology and Immunology, and Faculty of Agricultural Science, The University of British Columbia, Vancouver, B.C V6T 1Z3, Canada

Yeast-like cells of the corn smut pathogen *Ustilago maydis* mate to form the filamentous dikaryon that is capable of infecting corn plants. We found that the dimorphic switch from budding to filamentous growth was triggered by the presence of triglycerides supplied as corn oil (or other oils) or fatty acids (supplied as tweens) in the culture medium. The ability of the fungus to respond required components of the ras/MAPK and the cAMP/PKA signal transduction pathways that are known to mediate morphological changes in *U. maydis*. For example, a mutant with a defect in the regulatory subunit of protein kinase A (encoded by the *ubc1* gene) failed to form filaments in response to the lipid signals. Similarly, a mutant defective in a MAPK gene (*ubc3*) also failed to repond. An extracellular lipase activity was evident in culture supernatants during the morphological transition as determined by a turbidimetric enzyme assay. Glucose repressed the switch to filamentous growth in response to triglycerides and fatty acids, and inhibited the extracellular lipase activity in culture supernatants. It is possible that one or more of secreted lipases may contribute to the response of the fungus to triglycerides, and may ultimately contribute to virulence. Overall, these results provide the framework for a model for triglyceride/fatty acid signaling in *U. maydis* and establish a foundation for subsequent molecular genetic experimentation.

350. Insertional mutagenesis of *Fusarium graminearum* from rice in Korea. You-Kyoung Han[1], In Young Jang[1], Hun Kim[1], Sung-Hwan Yun[2], and Yin-Won Lee[1]. [1]School of Agricultural Biotechnology, Seoul National University, Suwon, 441-744, Korea,[2]Division of Life Sciences, Soonchunhyang University, Asan, Choongnam, 336-745, Korea

Fusarium graminearum is an important pathogen of cereal crops in many areas of the world causing head blight and ear rot of small grains. In addition to serious economic losses, this fungus produces mycotoxins, such as trichothecenes and zearalenone on diseased crops and has been a potential threat to human and animal health. Recently, it has been confirmed that *F. graminearum* was associated with epidemic of rice head blight occurred in Korea. More than 200 filed isolates of *F. graminearum* obtained from disease symptoms of rice in Korea were investigated for their pathogenicity against rice as well as other mycological characteristics. Meanwhile, genetic diversity of the *F. graminearum* population was evaluated by using amplified fragment length polymorphisms (AFLP). To massively identify pathogenesis-related genes from *F. graminearum*, two representative strains (SCKO4 from rice and Z03643 from wheat) were mutagenized using restriction enzyme-mediated integration (REMI). In total, 20,000 REMI transformants have been collected from the two strains. So far 60 mutants for several traits involved in disease development such as virulence, mycotoxin production, and sporulation have been selected from 2,000 transformants. Now, selected mutants of interest are genetically analyzed using a newly developed outcross method (See Jungkwan Lee et al poster). In addition, cloning and characterization of genomic DNA fragments flanking the insertional site in the genome of selected mutants are in progress.

351. Functional analysis of *CLPT1*, a RAB/GTPase gene from the bean pathogen *Colletotrichum lindemuthianum*. Piyawane Siriputthaiwan[1], Corentin Herbert[1], Alain Jauneau[2], Marie-Thérèse Esquerré-Tugayé[1] and Bernard Dumas[1]. 1: UMR5546 CNRS-UPS and 2: IFR 40 Pôle de Biotechnologie Végétale Castanet-Tolosan, France

During colonization of their host, phytopathogenic fungi secrete enzymes that degrade plant cell wall polymers. Since the secretory pathway could be a major control step for the production of these extracellular proteins, we have undertaken the cloning and the functional characterization of a Rab-GTPase from *C. lindemuthianum*, a filamentous fungi causing anthracnose disease on bean. Rab/GTPases are included in the Ras superfamily of GTPases and play a major role in the regulation of vesicle trafficking. Recently we have isolated a Rab gene from *C. lindemuthianum*, named *CLPT1* (*C. lindemuthianum* Protein Transport 1). *CLPT1* encodes a functional homologue of the yeast RAB/GTPase, Sec4p. To study more precisely the role of this protein, a *CLPT1* gene carrying a dominant-negative mutation (N123I) was expressed in *C. lindemuthianum*. Transgenic strains expressing this mutated gene accumulated large amount of vesicles randomly distributed in the fungal cells, were unable to produce extracellular enzymes and were non-pathogenic. However, they grew normally on synthetic media and differentiated appressoria

on glass surface. Altogether, these results showed that *CLPT1* plays a key role in pathogenesis being essential for the post-golgi vesicular transport allowing the secretion of extracellular enzymes.

352. Identification of *FDB1*, *FDB2*, and other *Fusarium verticillioides*genes expressed in response to BOA, a maize antimicrobial compound. Anthony E. Glenn and Charles W. Bacon. USDA, ARS, Russell Research Center, Toxicology & Mycotoxin Research Unit, Athens, GA

Maize produces antimicrobial compounds (DIMBOA, DIBOA, MBOA, and BOA) implicated in disease resistance and insect feeding deterrence. *Fusarium verticillioides*, the most common fungal pathogen associated with maize, has the physiological capacity to biotransform these compounds into non-toxic metabolites. While data suggest such biotransformation is not a major virulence factor, the metabolic capacity may enhance the ecological fitness of *F. verticillioides* in a cornfield environment. Genetic analyses showed at least two loci, *FDB1* and *FDB2*, are necessary for biotransformation. The biotransformation pathway for BOA is suggested to involve hydrolysis (Fdb1p) to produce 2-aminophenol, which is subsequently modified by addition of a malonyl group (Fdb2p) to produce *N*-(2-hydroxyphenyl) malonamic acid. If either gene is mutated, detoxification does not occur and the fungus cannot grow on BOA-amended medium. In an effort to molecularly characterize *FDB1* and *FDB2* as well as other genes involved in biotransformation, suppression subtractive hybridization (SSH) was used to target genes up-regulated in response to BOA. Among the clones identified, those with similarities to amidase and arylamine *N*-acetyltransferase were of particular interest, since these enzymes catalyze chemical modifications similar to those postulated for Fdb1p and Fdb2p. Genomic cosmid clones were identified for each using the respective cDNAs as probes. The putative amidase cosmid genetically complemented an *fdb1* mutation, while the putative*N*-malonyltransferase cosmid complimented an *fdb2* mutation. Thus, the proposed chemical modifications and the putative proteins involved are mutually supported. Also, these results demonstrate the utility of SSH for cloning genes previously identified by forward genetics.

353. Functional analysis of *Ustilago maydis* Ubc2, a putative novel adapter protein. Steven J. Klosterman, Alfredo D. Martinez-Espinoza, and Scott E. Gold. Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274.

The plant pathogenic fungus *Ustilago maydis* alternates between a haploid budding form and a dikaryotic filamentous form. Genes encoding proteins involved in the MAP kinase pathway that controls mating and morphogenesis have been identified previously by complementation of mutants that suppress the constitutively filamentous phenotype of a*uac1* (*Ustilago* adenylate cyclase) mutant. Thus these proteins were named Ubc (*Ustilago* bypass of cyclase) proteins. Three of the Ubc proteins share homology with MAP kinase cascade members that control mating and morphogenesis in other fungi. Another of these genes, *ubc2*, is a critical virulence factor and enocdes a protein which appears basidiomycete-specific in its overall structure. Ubc2 possesses four distinct protein interaction domains, indicating that Ubc2 is likely a novel adapter protein functioning in the MAP kinase pathway. To ascertain the role of the various protein interaction domains and to determine the functionally important amino acids within these domains of Ubc2, site-directed mutagenesis and complementation studies are being conducted. To gain further insight into the function of Ubc2, the yeast two-hybrid assay is being employed to identify interactions between Ubc2 and other proteins in the MAP kinase and potentially related pathways. The results of these studies will be discussed.

354. Molecular evolution of the *AVR-Pita* avirulence gene family in *Magnaporthe grisea.* Chang Hyun Khang[1], Seogchan Kang[1], and Barbara Valent[2]. [1] Dept. Plant Pathology, The Pennsylvania State University, University Park, PA 16802. [2] Dept. Plant Pathology, Kansas State University, Manhattan, KS 66506.

Magnaporthe grisea, the causal agent of the devastating rice blast disease, can infect many gramineous species. The host specificity of this fungus follows the gene-for-gene model. Deploying disease resistance (*R*) genes for disease control has had limited success, mainly due to the evolution of new races with the ability to overcome resistance. Considering its mainly asexual population structure, frequent variation of pathogen avirulence (*AVR*) genes likely underpins such evolutionary events. Therefore, understanding the mechanisms of variation and biological role of *AVR* genes is of importance for designing durable resistance. *AVR-Pita*, one of the *M. grisea AVR* genes, prevents the fungus from infecting the rice cultivars that express the *Pi-ta R* gene. *AVR-Pita* is located near a telomere and predicted to encode a 223 amino acid protein with similarity to metalloproteases. Southern blot analyses revealed

that *AVR-Pita* is a member of a widespread gene family in the *M. grisea* strains from various hosts including rice. Phylogenetic analyses and comparative study of their genome organization suggest that the *AVR-Pita* gene family consists of at least three members that have been duplicated and rearranged via recombination events, probably mediated by repetitive DNA elements flanking the gene family. The conservation of AVR-Pita protein structures within *M. grisea* populations and the presence of strains lacking any characterized family members suggest that members of this family may function as a host-specific fitness factor.

355. Characterization of a gene cluster for host-specific AAL-toxin biosynthesis in the tomato pathotype of *Alternaria alternata*. Hajime Akamatsu, Hiroshi Otani and Motoichiro Kodama. Laboratory of Plant Pathology, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan

AAL-toxins are host-specific toxins produced by *Alternaria alternata* tomato pathotype, the causal agent of Alternaria stem canker of tomato. AAL-toxins and fumonisins of the maize pathogen *Gibberella moniliformis* are structurally related to sphinganine and are termed sphinganine-analogue mycotoxins. Since AAL-toxins are polyketide-derived compounds similar to fumonisins, we have cloned several polyketide synthase (PKS) gene fragments from the tomato pathotype of *A. alternata* by PCR. Gene targeting with a vector harbouring a PKS sequence specific to the tomato pathotype created AAL-toxin-minus mutants that lost virulence to the susceptible tomato. Analysis of flanking sequences of the PKS fragment from a genomic library of the tomato pathotype revealed a Type I PKS gene of 7.8 kb in length, designated *ALT1*. Flanking *ALT1*, several genes were identified which constitute an AAL-toxin biosynthetic (*ALT*) gene cluster, and some of these genes resembled those found in fumonisin biosynthetic (*FUM*) gene cluster. The predicted products of the genes in the cluster were similar to fungal Type I PKSs, cytochrome P450 fusion proteins, alcohol dehydrogenases, aminotransferases, ABC transporters and longevity assurance factors. However, the order of the genes in the *ALT* gene cluster was different from that of the *FUM* gene cluster. The *ALT* gene cluster reside on a 1.0 Mb conditionally dispensable chromosome found only in the AAL-toxin-producing strains of *A. alternata* and homologues of the genes were not detected in nonpathogenic strains of *A. alternata*. This suggests that the *ALT* gene cluster may have been acquired by horizontal gene transfer and provides a possible mechanism whereby new pathotypes could arise in nature.

356. The phosphoinositide-specific phospholipase C gene, *MPLC1*, of *Magnaporthe grisea* is required for fungal development and plant colonization. Hee-Sool Rho, and Yong-Hwan Lee, School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

Magnaporthe grisea, the casual agent of rice blast, forms an appressorium to penetrate its host. Much has been learned about environmental cues and signal transduction pathways, especially those involving cAMP and MAP kinases, on appressorium formation during the last decade. More recently, pharmacological data suggest that calcium/calmodulin-dependent signaling system is involved in its appressorium formation. To determine the role of phosphoinositide-specific phospholipase C (PI-PLC) on appressorium formation, a gene (*MPLC1*) encoding PI-PLC was cloned and characterized from *M. grisea* strain 70-15. Sequence analysis showed that *MPLC1* has all five conserved domains present in other phospholipase C genes from several filamentous fungi and mammals. Null mutants (*mplc1*) generated by targeted gene disruption exhibited pleiotropic effects on conidial morphology, appressorium formation, fertility and pathogenicity. *mplc1* mutants developed nonfunctional appressoria and are also defective in infectious growth in host tissues. Defects in appressorium formation and pathogenicity in *mplc1* mutants were complemented by a mouse PLCdelta-1 cDNA under the control of the *MPLC1* promoter. These results suggest that cellular signaling mediated by *MPLC1* plays crucial and diverse roles in development and pathogenicity of *M. grisea*, and functional conservation between fungal and mammalian PI-PLCs.

357. Identification of race-specific avirulence genes in *Phytophthora infestans* by transciptional profiling. Rays H.Y. Jiang[1], Guo Jun[1,2] and Francine Govers[1]. [1]Laboratory of Phytopathology, Wageningen University, and Graduate School Experimental Plant Sciences, Wageningen, The Netherlands.[2]Institute of Vegetable Crops and Flowers, Chinese Academy of Agricultural Sciences., Beijing, China.

Phytophthora infestans is a destructive oomycete pathogen causing potato late blight worldwide. Genetic analyses of potato and *P. infestans* have demonstrated that in this pathosystem, monogenic resistance mediated by resistance (*R*) genes, is based on a gene-for-gene interaction. Our aim is to clone and characterise avirulence (*Avr*) genes in *P. infestans*. Previously, we constructed high density linkage maps of two regions carrying *Avr* genes (van der Lee et

al. 2001 Genetics 157: 949-956). Currently, cDNA-AFLP analysis is performed on F1 progeny of the mapping population. Twenty-one isolates with different avirulence phenotypes have been selected and mRNA has been isolated from the stage that *Avr* genes are most likely to be expressed, i.e., the germinating cyst stage. Based on the transcriptional profiles we can identify genes that are differentially expressed in different races and this may lead to identification of *Avr* genes in *P. infestans*.

358. Effects on virulence of the tomato pathogen *Cladosporium fulvum* by RNAi-mediated silencing of avirulence genes. Bas Brandwagt[1,2], Maarten de Kock[2], Matthieu Joosten[1] and Pierre de Wit[1]. Laboratories of Phytopathology[1] and Plant Breeding[2], Wageningen University, The Netherland

The interaction between the biotrophic fungus *Cladosporium fulvum* and tomato (*Lycopersicon esculentum*) complies with the gene-for-gene model. Fungal growth is restricted to the extracellular spaces of tomato leaves, where *C. fulvum* secretes avirulence (AVR) proteins. AVR proteins are strain-specific and elicit a hypersensitive response in tomato genotypes with the corresponding resistance (*Cf*) genes. AVR proteins secreted by all known strains of *C. fulvum* are called extracellular proteins (ECPs) and are presumed to be virulence factors. Our goal is to study the function of the AVRs and ECPs in establishing or aborting infection of tomato by *C. fulvum.* We aimed to silence the (a)virulence genes of *C. fulvum* by transformation with constructs inducing RNA interference (RNAi). First, we proved that RNAi-mediated silencing in *C. fulvum* was possible for the *Uid*A (GUS), *EGFP* and hydrophobin marker genes. Subsequently, two highly virulent *C. fulvum* strains were independently transformed with *Ecp1*, *Ecp2*, *Ecp4*, *Ecp5* or *Avr4E* RNAi constructs. The*Ecp2* and *Avr4E* RNAi transformants could colonise Cf-ECP2 and Cf-4E tomato plants through the absence of the ECP2 and AVR4E proteins, respectively. These observations prove that the reduction of avirulence gene expression by RNAi is sufficient to prevent resistance responses in tomato plants with the corresponding *Cf* gene. The *Ecp1*, *Ecp4* and *Ecp5* RNAi transformants, however, were still avirulent on plants with the corresponding *Cf* genes. We are now further analysing this phenomenon. In conclusion, RNAi in *C. fulvum* can be used as a versatile tool to determine virulence functions of avirulence proteins in the interaction between *C. fulvum* and tomato.

359. The role of the mitochondrial Mrb1 protein in pathogenic development of *Ustilago maydis*. Miriam Bortfeld, Kathrin Auffarth and Christoph W. Basse. Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse, 35043 Marburg/Lahn, Germany

The smut fungus *Ustilago maydis* causes tumor development in infected maize plants. The constitutively expressed *Ustilago maydis mrb1* gene encodes a protein with significant similarity to mitochondrial, acidic proteins of the p32 family known from different eukaryotic organisms. Mitochondrial localization of Mrb1 was demonstrated and the mitochondrial targeting sequence was delineated. Compatible *U. maydis mrb1*-null mutants derived from strains FB1 and FB2 were able to mate, however, the resulting dikaryotic hyphae were severely attenuated in pathogenicity. Despite their abilities to develop appressoria-like structures on maize leaf surfaces and to penetrate throughout epidermal layers, ramification and proliferation within infected tissue as well as the capacity to induce tumor development were drastically reduced compared to wild-type hyphae. Intriguingly, pathogenicity of haploid, solopathogenic *U. maydis* strains derived from FB1 was not affected by the deletion of *mrb1*, whereas *mrb1* was required for pathogenicity of haploid, solopathogenic FB2 strains, suggesting that FB2 specific factors compromise pathogenicity in the absence of Mrb1.

360. Sensing, signalling and stress in the barley powdery mildew fungus. Ziguo Zhang, Catherine Henderson, Gemma Priddey, Emma Perfect and Sarah Gurr: Department of Plant Sciences, University of Oxford, OX1 3RB, UK.

Blumeria graminis is the causal agent of barley powdery mildew disease. Infection is spread by asexual conidia, which, on contact with the leaf surface, undergo a complex and highly regulated programme of development. Conidia germinate and produce a short primary germ tube followed by a second formed germ tube which elongates, swells and produces a specialised, hooked infection structure, the appressorium. *B. graminis* is an obligate biotroph; it cannot be grown axenically and consequently, tissue for experiments is limiting. We have described a range of techniques to assess how *B. graminis* perceives, integrates and relays signals for morphogenesis up to the point of penetration. Previously, our work demonstrated that both physical properties of the leaf surface, such as hydrophobicity, and cuticle-derived chemicals promote *B. graminis* differentiation. But 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. Hitherto, we have identified other genes involved in signal transduction and cell integrity pathways in *B. graminis*, notably PKC, MAPKinases and cell wall genes. We will discuss this work, considering also the pathogen's management of host-derived oxidative stress during development and penetration.

361. Characterisation of the MPS1 MAP Kinase signalling pathway in *Magnaporthe grisea*. Zac Cartwright and Nicholas J. Talbot. School of Biological Sciences, University of Exeter, EX4 4QJ, United Kingdom.

Fungal plant pathogens have evolved diverse methods of gaining entry to their particular host plant tissue. One fungal pathogen that makes use of mechanical entry is the ascomycete *Magnaporthe grisea*. Once present on a leaf surface the spore quickly attaches to the hydrophobic leaf surface by secreting an adhesive from within the spore apex. The spore produces a germ tube, and after four hours, differentiates into an appressorium. Penetration into the leaf epidermis occurs when the pressure within the appressorium reaches an estimated 8.0 MPa, causing a penetration peg to rupture the cuticle. The mitogen-activated protein kinase (MAPK) MPS1 is involved in an uncharacterised pathway in the pathogenicity of *M. grisea.* It is known that mutants lacking the MPS1 gene are able to produce appressoria, but are unable to penetrate the plant cuticle and are therefore non-pathogenic. MPS1 has homologues in a variety of other fungal species; SLT2/MPK1 in *Saccharomyces cerevisiae*, MKC1 in *Candida albicans* and CPMK2 in *Claviceps purpurea*. SLT2 in yeast is a component in the pathway that regulates maintenance of cell wall integrity. The aim of this project is to investigate the biological function of the MPS1 MAP kinase gene in *M. grisea*. Studies to identify genes regulateded by the MPS1 pathway have been undertaken and preliminary analysis indicates differential regulation of a glycogen synthase kinase-encoding gene in mps1 mutants. Results of differential expression studies will be presented.

362. Fungal H2O2 scavenger activity influences pathogenesis in the true obligate barley powdery mildew pathogen. Ziguo Zhang, Catherine Henderson & Sarah J. Gurr, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK

The barley powdery mildew, *Blumeria graminis*, is exposed to acute host-derived oxidative stress at critical times during germling morphogenesis, notably at the primary and appressorium germ tube (PGT, AGT) stages of differentiation. To evaluate the antioxidant capacity of this fungus, in particular to assess how Bgh protects itself against damage caused by the accumulation of H2O2 and if fungal catalase contributes towards pathogenicity, the Bgh catB gene was cloned and characterised. An anti-CATB antibody revealed an intense circle of immunofluorescence at the host-pathogen interface at the AGT tip and within the halo area surrounding the host papilla, whilst Diaminobenzidine (DAB) revealed H2O2 accumulation in the papilla. RT-PCR profiling of catB transcript activity, alongside other genes implicated in the management of oxidative stress (catalase-peroxidase, cpx; glutathione peroxidase, gpx and superoxide dismutase, sod1), revealed enhanced numbers of catB transcripts at mature PGT and AGT stages of differentiation in a susceptible host. Moreover, comparison of susceptible (S) barley cv Pallas with its resistant counterpart carrying the papilla-based resistance gene (R) mlo5, P22, revealed intense DAB staining in the papilla and halo beneath the PGT in both S and R lines, but rare staining of the papilla and halo beneath AGTs in the S interaction, provoking speculation that Bgh catalase scavenger activity influences pathogenicity in the barley powdery mildew fungus.

363. The mitogen-activated protein kinase Tvk1 from *Trichoderma virens* regulates mycoparasitism related genes and conidiation. Artemio Mendoza-Mendoza[1], Darlene Grzegorski[2], María J. Pozo[2], Pedro Martínez[1], Juan García[2], Vianey Olmedo-Monfil[1], Carlos Cortés[1], Charles Kenerley[2] and Alfredo Herrera-Estrella[1].[1]Department of Plant Genetic Engineering, Centro de Investigación y Estudios Avanzados. Unidad Irapuato. Apartado Postal 629. 36500, Irapuato, Guanajuato., México.[2]Department of Plant Pathology & Microbiology, TEXAS A&M University. College Station, TX 77843, USA.

Trichoderma virens has a potent antagonic activity against a broad range of phytopathogenic fungi. In the parasitic interaction *Trichoderma* produces lytic enzymes that have been associated with penetration of the host. In phytopathogenic fungi, the production of lytic enzymes that degrade the cell wall of the host is regulated through a mitogen-activated protein kinase (MAPK) pathway. A MAP kinase-encoding gene from *T.virens*, named *tvk1*, was

cloned and its physiological role was assessed. Cell growth, conidiation and the expression of mycoparasitic related genes (MRGs) were examined in *tvk1* disruptants. Null mutants showed a reduction in aerial growth and production of spores when grown in solid medium. On the other hand a massive production of conidia was achieved in submerged cultures, suggesting that this kinase can act as a negative regulator of conidiation under this condition. During simulated mycoparasitism or direct confrontation with a host (*Rhizoctonia solani*), the mutants showed a clear augment in the level of expression of all MRGs tested when compared with the wild type strain. The increased transcription of lytic enzyme-encoding genes correlated with an increase in protein secretion as measured by *in gel* activities. Finally, biocontrol activity assays showed that *tvk1* null mutants are more effective in disease control than the wild type strain.

364. Possible function for a F-box protein in pathogenicity of *Fusariumoxysporum* f. sp. *lycopersici*. Roselinde Duyvesteijn, Petra M. Houterman, Martijn Rep, and Ben J. C. Cornelissen. University of Amsterdam, Faculty of Science, Swammerdam Institute for Life Sciences, Plant Pathology, Amsterdam, The Netherlands

Fusarium oxysporum f. sp. *lycopersici* is a soil-borne pathogen that infects tomato. The fungus invades the plant via the roots and subsequently colonizes the xylem vessels of the stem. Ultimately, the sap stream is blocked and the plant wilts. We used insertional mutagenesis to identify, isolate and characterize genes involved in pathogenesis. From among 398 transformants, 19 mutants were identified that showed reduction in or complete loss of pathogenicity but normal growth on agar plates. Plasmid rescue was used to obtain flanking regions of the inserted plasmid from mutant N40 which has complete loss of pathogenicity. Approximately 3 kb *Fusarium* DNA was retrieved and used to screen a BAC-library in order to obtain the wild type locus. Sequence analyses revealed that the insertion point was in a gene for a 527 AA protein containing an F-box domain. F-box proteins are involved in the ubiquitination machinery of the cell. The protein has homology with a protein from*Magnaporthe grisea*, a plant-pathogenic fungus on Rice, and one from *Neurospora crassa*, a non-pathogenic fungus. The genomic regions containing the genes for the F-box proteins showed synteny over at least 12 kb in all three fungi. A transcript for the F-box protein was found in a cDNA library made of tomato plants infected with*Fusarium*, indicating that the gene is active during infection. Complementation and knockout studies will be done to determine the relation between the insertion and the loss of pathogenicity.

365. ABC transporters of *Mycosphaerella graminicola* involved in pathogenesis and multidrug resistance. Lute-Harm Zwiers, Ioannis Stergiopoulos and Maarten A. De Waard Laboratory of Phytopathology, Department of Plant Sciences, Wageningen University, Wageningen, the Netherlands

ATP-binding cassette (ABC) transporters are membrane-bound proteins that in plant pathogenic fungi are implicated to act as virulence factors by providing protection against plant defense compounds or by secreting pathogenicity factors. Moreover, ABC transporters are involved in the establishment of multi-drug resistance (MDR) and thus impose a serious threat to the successful control of fungi with antimycotics. We are interested in the role of ABC transporters in *Mycosphaerella graminicola*, the causal agent of septoria tritici leaf blotch of wheat. Five single copy ABC transporter genes (*MgAtr1* to *MgAtr5*) have been cloned and characterised from this pathogen. The function of these genes was investigated with knockout mutants, generated by PEG- and *Agrobacterium*-mediated transformation. All transformants were tested for virulence on wheat seedlings. Furthermore, the role of these transporters in MDR was studied by complementation of *Saccharomyces cerevisiae* mutants with the *M. graminicola* ABC transporter genes and by testing the sensitivity of ABC transporter knockout mutants of *M. graminicola* to natural toxic compounds, xenobiotics, and antagonistic bacteria. Our results show that MgAtr4 plays a role in virulence, whereas other ABC transporters provide protection against fungicides, plant metabolites and antibiotics produced by antagonistic bacteria. Therefore, the data presented suggest that ABC transporters contribute to the competitive ability of *M. graminicola*.

366. Transcript profiling of the fungus *Monacrosporium haptotylum* during infection of the nematode *Caenorhabditis elegans*. Tunlid, A., Fekete, C., Tholander, M., Ahren, D, Friman, E. and Johansson, T. Department of Microbial Ecology, Lund university, Lund, Sweden

Monacrosporium haptotylum infects nematodes using adhesive knobs, which are developed on branches of vegetative mycelium. To identify genes and metabolic pathways that are expressed during development of traps and infection of nematodes, we have analyzed 7113 expressed sequence tags from four different cDNA libraries,

vegetative mycelium, knobs, and knobs infecting the nematode *Caenorhabditis elegans* for 4h and 24 h, respectively. The ESTs were clustered into 2729 unigenes (contigs) representing 2729 putatively unique transcripts. Approximately 400 of these unigenes were of nematode origin. Approximately 23-31 % of all ESTs displayed significant similarities to sequences found in the GenBank database and this information was used for functional and metabolic annotations. Only 10.4 % of the contigs contained ESTs from more than one cDNA library, thus the patterns of genes expressed in the three libraries were significantly different. To obtain further information on the expression levels of the ESTs, the total set of unigenes were spotted on a cDNA array. Data on the patterns of expression of these genes will be presented.

367. Mating, conidiation and pathogenicity of *Fusarium graminearum*, the main causal agent of the head blight disease of wheat, are regulated by the MAP kinase Gpmk1. Nicole J. Jenczmionka, Frank J. Maier, Anke P. Loesch, and Wilhelm Schaefer. University of Hamburg, Institute of General Botany, Department of Molecular Phytopathology and Genetics (AMP III), Ohnhorststr. 18, D-22609 Hamburg, Germany.

Up to date only very little is known about the molecular infection mechanisms of the head blight pathogen of wheat, *Fusarium graminearum* (teleomorph *Gibberella zeae*). Here we report on the isolation and characterization of the Fus3 / Pmk1 MAP kinase homologue Gpmk1 from *F. graminearum*. Transformation-mediated gene disruption was used to create strains with a non functional *Gpmk1* coding sequence. RT-PCR proved the absence of the corresponding mRNA. The deletion mutants exhibit a reduced conidia production and are sexually sterile. Inoculation into wheat spikeletts demonstrate that the D*Gpmk1* mutants are fully apathogenic. Therefor, this MAP kinase is involved in the regulation of several important steps of the fungal life cycle, including pathogenicity.

368. Functional characterization of the transcription factor *Fopta1* in strains of *Fusarium oxysporum* f.sp. *phaseoli*. B. Ramos, A. García-Sánchez, A.P. Eslava and J.M. Díaz-Mínguez. Área de Genética, Centro Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca, 37007 Salamanca, Spain.

Fusarium oxysporum f.sp. *phaseoli* J.B. Kendrick & W.C. Snyder is the fungus wich causes Fusarium wilt, a serious disease of common bean (*Phaseolus vulgaris* L.). There are over 120 formae speciales described capable of causing this disease. It shows a wide range of infection as species and host specificity as formae speciales, that makes it an attractive model for the study of the molecular interactions involved in pathogenicity and/or virulence. We have isolated the *Fopta1* gene (*F. oxysporum* f.sp. *phaseoli* transcription activator), belonging to the GAL4 family of transcription factors. Copies of this gene are present both in pathogenic and nonpathogenic strains of *F. oxysporum* f.sp.*phaseoli*, but only the copies in highly virulent strains seem to be fully functional. We have analized the differences in the promoter region to study how some minor nucleotide changes in the TATA box can drastically reduce the expression of *Fopta1* in weakly virulent and nonpathogenic strains. Previously we had detected the expression of *Fopta1* by RT-PCR both in mycelium of highly virulent strains grown in vitro and in bean plants inoculated with these strains. Here we report the analysis of expression of *Fopta1* in the course of the infection process in bean plants by means of real-time PCR, in comparison with a constitutive expressed gene coding for beta-tubulin. Also, we show the analysis of gene inactivated mutants obtained by using a transformation procedure based on *Agrobacterium tumefaciens*. This research was supported by grant AGL 2001-2052 (Ministerio de Ciencia y Tecnología of Spain). Brisa Ramos was the recipient of a fellowship from the INIA (Ministerio de Ciencia y Tecnología of Spain) and Asunción García-Sánchez was the recipient of a fellowship from Caja Rural de Salamanca (Castilla y León, Spain).

369. Disruption of the homoaconitase gene of the fungal barley pathogen *Pyrenophora teres* results in lysin auxotrophy, disturbed conidiation and strongly reduced virulence. Karen Sonnenberger, Frank J. Maier, and Wilhelm Schaefer. University of Hamburg, Institute of General Botany, Department of Molecular Phytopathology and Genetics (AMP III), Ohnhorststr. 18, D-22609 Hamburg, Germany.

The lysin biosynthesis pathways of fungi and plants differ greatly. Therefor, fungal enzymes involved in lysin biosynthesis may serve as targets for new fungicides. We cloned the homoaconitase gene *lys4*) from the fungal barley pathogen *Pyrenophora teres*. Transformation-mediated gene replacement was used to create strains which lack a major part of the *lys4* coding sequence. RT-PCR proved the absence of the homoaconitase mRNA. Homoaconitase enzyme activity was measured in a crude extract of mycelia grown in complete medium. The D *lys4* mutants exhibit a 65 % reduced activity in comparison to wild type. D *lys4* mutants are unable to grow *in-vitro* on

minimal medium, whereas wild type like growth can be restored by addition of lysin to the medium. Conidiation of the D*lys4 P. teres* strains was reduced to less then 10%, even under optimal conditions. Pathogenicity studies of D *lys4*. teres strains show that they are greatly reduced in virulence and unable to colonize barley leaves beyond the point of inoculation. For the first time, we could show that lysin prototrophy is necessary for virulence of a phytopathogenic fungus.

370. Analysis of a non ribosomal peptide synthase gene from *Alternaria brassicae* and flanking genomic sequences. Thomas Guillemette, Babu Subramanian, Adnane Sellam, Philippe Simoneau UMR PaVé N77, Faculté des Sciences, 2 Bd Lavoisier, 49045 Angers cedex, France

Alternaria brassicae, a necrotrophic seed-borne pathogen of crucifers, produces host-specific toxins called destruxins. These metabolites are cyclic depsipeptides that may be synthesized by the fungus via a non ribosomal route using a multifunctional modular enzyme called non-ribosomal peptide synthase (NRPS). A 22 kbp NRPS gene was identified in *A. brassicae* by screening a cosmidic genomic library with an homologous PCR probe obtained with degenerate primers. This gene encodes a protein of ca. 790 kDa that contains four amino-acid activation, three epimerization, six condensation and six thiolation modules. The modular architecture of this peptide synthase suggests the presence of at least one additional NRPS gene in the *A. brassicae* genome. Approximatively 4.5 kbp upstream the NRPS start codon, a gene encoding an ABC-transporter (MRP type) was found. In addition, a dicarboxylic acid permease gene and an ORF similar to the *Bys1* gene of the dimorphic fungus *Blastomyces* are present immediately downstream the NRPS stop codon. Structural analysis of these genes and corresponding proteins as well as results of expression studies will be presented.

371. Enzyme characteristics of *Candida albicans* secreted lipases LIP1, LIP4, and LIP10. Siegfried Salomon, Inga M. Melzer, Christian A. Haase, Frank Stehr, and Wilhelm Schaefer. University of Hamburg, Institute of General Botany, Department of Molecular Phytopathology and Genetics (AMP III), Ohnhorststr. 18, D-22609 Hamburg, Germany.

The human pathogen *Candida albicans* is able to cause several different types of infections such as oral, vaginal, or systemic candidosis. Various virulence factors have been suggested to be important during different types of *C. albicans* infections. These factors include the yeast-to-hyphal transition, adhesion factors, or surface hydrophobicity, phenotypic switching, molecular mimicry, and the secretion of hydrolytic enzymes. While secreted aspartic proteinases and phospholipases are well characterized, the role of other secreted hydrolytic enzymes such as esterases and lipases as virulence factors is unknown. Lipolytic activity enables*C. albicans* to grow on lipids as the sole carbon source. Southern blot analysis and screening of the sequence data from the *C. albicans* genome project revealed a family of lipase genes with at least ten members. The ORFs of the ten lipase genes consist of between 1281 and 1416 bp and encode highly similar proteins with up to 80 % identical amino acid sequences. For detailed biochemical characterization of lipase 1 (LIP1), 4 (LIP4), and 10 (LIP10), the enzymes were expressed functionally in the methylotrophic yeast *Pichia pastoris.* After transformation and selection, the heterologous expressed enzymes were tested for pH and temperature optimum, substrate specificity, and sensitivity against drugs which inhibit lipases. Our data clearly demonstrated that LIP1, LIP4, and LIP10 shows distinguished activities in spite of their high sequence homology.

372. Pathogenesis of *Mycosphaerella graminicola* : two complementary approaches for the identification of molecular determinants. A. Cousin, M. Dufresne and T. Langin. Laboratoire de Phytopathologie Moléculaire, Institut de Biotechnologie des Plantes, Université Paris-Sud, France.

Septoria leaf blotch of wheat caused by *Septoria tritici* (teleomorph, *Mycosphaerella graminicola*) is a serious foliar disease that becomes increasingly important in all temperate regions of the world. This fungus penetrates the leaf through stomatal openings without the differentiation of any penetration structures. The infection cycle of *M.graminicola* is relatively well known at the cytological level, however the molecular basis of pathogenesis is poorly understood. In order to identify pathogenicity genes, different strategies have been developed in our laboratory. The first one consists in random insertional mutagenesis using a transforming plasmid. 195 stable hygromycin-resistant transformants of *M.graminicola* were obtained and screened for loss of pathogenicity using two infection assays, (i) on detached leaves and (ii) on seedlings. Among 11 mutants showing a reduced ability to induce blotch symptoms, two have been studied in more details : A18, a non pathogenic mutant and D22 showing a

great reduction of pathogenicity. Molecular analysis and more detailed phenotypic characterization of these two strains will be presented. The second strategy consists in a candidat approach. Based on homology with the pmk1 gene of *M.grisea* encoding a MAP kinase essential for appressorium formation and invasive growth, the mgmk1 gene from *M.graminicola* encoding a putative PMK1-like MAP kinase was cloned. The gene is present in the genome as a single copy. In order to assess the role of MgMK1 in the life cycle of *M.graminicola*, targeted gene disruption is currently conducted. Studies of mutants carrying an interrupted copy of the gene will be performed. Is MgMK1 essential for plant infection? May MAP kinase pathway be widely conserved in pathogenic fungi for regulating infection?

373. Transcriptome-like approach to study global and fine appressorium development and functionality in Colletotrichum lindemuthianum. R. Lauge, C. Veneault and *T. Langin*. Laboratoire de Phytopathologie Moléculaire, Institut de Biotechnologie des Plantes, Université Paris-Sud, France.

Colletotrichum lindemuthianum is the causal agent of anthracnose on common bean. This ascomycete is an interesting plant pathogen model, as it displays a hemibiotrophic cycle and differentiates several well-defined infection structures (appressorium, infection vesicle, primary hyphum, secondary hyphum) on its host. We have used a cDNA library to initiate a transcriptome-like approach on the different development stages of its infection cycle. 5000 independent clones have been archived, of which half have been spotted on filter replicates for expression studies. The setup of a method for in vitro differentiation of appressoria has allowed us to start the study of specific genes expression during this critical step for penetration. Clones presenting a differential expression have been identified and analysed. Among others, cla1 the *C. gloeosporioides* cap5 orthologue (unknown function) and cla3 an ubiquitin encoding gene were found upregulated, and. cla2 the yeast DSK2 orthologue (mitosis control via requirement for spindle body duplication) was found downregulated during appressorium development, respectively. Using this method, as well as SSH, we have also identified genes displaying a differential expression between wild-type appressorium and appressorium obtained from the clk1 kinase mutant which is blocked at the penetration step (Dufresne et al., 1998). Such genes, named clak for *C. lindemuthianum* appressorium kinase, could correspond to targets of the signal transduction pathway that CLK1 is part of. Possible involvement of these genes in appressorium development and/or functionality will be presented, as well as new genes that are under characterization.

374. Signalling in the interaction *Claviceps purpurea* and rye. Jan Scheffer and Paul Tudzynski. Institut für Botanik, Westfälische Wilhelms-Universität Münster, Germany.

Claviceps purpurea is a common phytopathogenic ascomycete which colonizes only grass florets. This interaction represents an interesting fungal model for oriented growth comparable e.g. to the guidance of axons to neural synapses in animals. Our main interest is to identify signalling cascade components involved in directed growth during infection. Strong candidates are those known to be essential for the pathogenicity of *Claviceps*, such as the cpmk2 MAP kinase (Mey et al. 2002). Deletion mutants invade the host tissue but they are not able to tap the vascular tissue. These mutants are a valuable tool for the identification of other signal chain components mediating directed growth. Further interesting genes possibly involved in directed and/or oriented growth are homologues to COT1, a Ser/Thr protein kinase responsible for hyphal elongation in *Neurospora crassa* and to CDC42 and Ras, small G proteins involved in the mediation of cell polarity. The influence of these genes in the orientation of *C. purpurea* is to be investigated. Another approach for the isolation of genes involved in oriented growth is the creation of an insertional mutant library based on the Agrobacterium-mediated T-DNA-transfer. Mutants with impaired growth can be identified easily in an in vitro system for cultivation and infection of rye ovaries, which has been established recently.

375. Gene regulation by the histone deacetylase Hdc1, and the identification of genes important for pathogenesis in *Cochliobolus carbonum*. Jennifer A.Bieszke[1], Johannes Galehr[2], Paolo Amedeo[3], Olen C. Yoder[3] and Jonathan D. Walton[1]. [1]MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA. [2]Department of Molecular Biology, University of Innsbruck, Innsbruck Austria. [3]Torrey Mesa Research Institute, 3115 Merryfield Row, San Diego, CA 92121, USA.

Hdc1 is a histone deacetylase important for pathogenesis of *Cochliobolus carbonum* on maize. A null *hdc1* mutant of *C. carbonum* has reduced virulence and down-regulation of genes encoding cell wall degrading enzymes (CWDEs) implicated in pathogenesis (Baidyaroy *et al.*, 2001, Plant Cell 13:1609). Two approaches are being used

to determine if Hdc1 has a direct role in transcriptional regulation of CWDEs during pathogenesis. The first is to characterize the global acetylation state in the *hdc1*mutant as compared to wild type using quantitative protein blot analysis with antibodies specific to different acetylated forms of histones. The second approach is to analyze differences in the acetylation states among CWDE promoters using chromatin immunoprecipitation (ChIP). In addition to characterizing the mechanism of gene regulation by Hdc1, a second objective is to identify additional genes whose expression is affected by *HDC1* or by*ccSNF1* (which also causes reduced transcription of CWDE genes and reduced virulence; Tonukari *et al.*, 2000, Plant Cell 12:237). To do this an Affymetrix GeneChip array with ~20,000 DNA sequences of *C. heterostrophus*, which is also a foliar pathogen of maize, was probed with RNA from wild type or from *hdc1* or *ccsnf1* mutants. All strains were grown under conditions that either repress or induce CWDE gene expression. Under inducing conditions, ~600 genes were regulated differentially in wild type. Further analysis has differentiated the regulation of some of these genes in the two mutants as well as identified a subset of these genes that appears to be co-regulated by *HDC1* and *ccSNF1*, whereas regulation of other genes differs between the two mutants. Genes identified by GeneChip analysis will be confirmed by RNA blotting, then tested for a role in growth and pathogenesis by targeted gene disruption.

376. Pathogenicity factors and host responses in the *Heterobasidion annosum* conifer pathosystem. Jan Stenlid, Åke Olson, Magnus Karlsson, Mårten Lind and Fred Asiegbu. Department of Forest Mycology and Pathology, University of Agricultural Sciences, SWEDEN

The presentation will provide information on factors that are important to determine the success of *Heterobasidion annosum* as a pathogen of conifers. Important basic traits include ample spore production, colonisation and growth in host tissue including ability to attach to cell surfaces, to degrade macromolecules, to produce toxins, to handle low oxygen tension, and to detoxify host defence. Recent EST projects have indicated induction of a range of proteins including hydrophobins, mitochondrial proteins and proteins involved in signal transduction pathways. Infection experiments with defined strains of *H. annosum* have shown the importance of nucleus-mitochondria interaction for the expression of virulence. A genetic map is constructed for positioning of the pathogenicity factors. A search for host genes involved in the interaction with *H. annosum*. Following challenge with the pathogen, expressed genes in *Pinus sylvestris* has been hybridised to microarrays of *P. taeda*genes. A range of induced responses have been identified. Future work will focus on characterising early responses and to compare with the reactions of the host to saprotrophs and mycorrhizal fungi.

377. Abscisic acid biosynthesis in *Botrytis cinerea*. Verena Siewers and Paul Tudzynski. Institut fuer Botanik, Westfaelische Wilhelms-Universitaet Muenster, Germany

Botrytis cinerea causes the grey mould disease in more than 200 plant species. Like several other phytopathogenic fungi, *B. cinerea* has been shown to produce different kinds of phytohormones in axenic culture. Although the impact of this biosynthetic capacity on host-parasite interaction is still unclear, it has been suggested that production of the plant hormone abscisic acid (ABA) is supporting the infection process. Our aim is to investigate the role of fungal ABA by cloning genes encoding enzymes of the biosynthetic pathway, studying their expression and analysing the pathogenicity of deletion mutants. In plants, ABA is derived from cleavage products of carotenoids. We could exclude the presence of a similar pathway in *B. cinerea*, as inhibition of carotenoid biosynthesis with diphenylamine (DPA) and inactivation of a putative gene of this pathway did not affect the ability of the fungus to produce ABA. A group of enzymes probably involved in fungal ABA biosynthesis are P450 monooxygenases. Therefore, about 20 different P450 monooxygenase encoding genes of *B. cinerea* were cloned and characterized. Two of them (P450-12, P450-16) were found to be differentially expressed during ABA production. Gene deletion of P450-12 had no influence on the synthesis of the plant hormone. In another approach to investigate the possible role of P450 monooxygenases the cytochrome P450 oxidoreductase encoding gene (cpr) was isolated and deleted.

378. Isolation and characterization of *Fusarium graminearum* mutants compromised in mycotoxin production and virulence. Frances Trail [1], Martin Urban [2], Iffa Gaffoor, [1], Ellie Mott [3], Corrie Andries [1] and Kim Hammond-Kosack [2]. [1]Departments of Plant Biology and Plant Pathology, Michigan State University, East Lansing MI 48824, USA. [2] Plant-Pathogen Interactions Division, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK. [3] Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge, CB2 3EA, UK.

A population of random plasmid insertion *F. graminearum* mutants, derived from the strain PH-1, (n=1170) was screened for their ability to cause head blight disease of wheat. Nine disease attenuated *Fusarium*, *daf* mutants were recovered. Each *daf* mutant differed in its ability to (a) cause disease symptoms on the head and stem bases of wheat plants, (b) affect grain fill and (c) synthesize trichothecene mycotoxins. Additional characteristics of each *daf* mutant will be presented.

379. Expression Analysis of the *Candida albicans* Lipase Gene Family During Experimental Infections and in Patient Samples. Frank Stehr[1], Marianne Kretschmar[2], Birgit Maehnss[3], Karsten Neuber[3], Bernhard Hube[1,4], and Wilhelm Schaefer[1]. [1] University of Hamburg, Institute of General Botany, Department of Molecular Phytopathology and Genetics (AMP III), Ohnhorststr. 18, D-22609 Hamburg, Germany, [2]Institute of Medical Microbiology and Hygiene, Faculty of Clinical Medicine, Mannheim, [3]University Hospital Hamburg-Eppendorf, Department of Dermatology and Allergology, [4]Robert Koch-Institute, Berlin.

Secreted lipases of *Candida albicans* are encoded by a gene family with at least ten members (*LIP1-LIP10*). The role of these putative virulence factors in pathogenicity still needs to be elucidated. Therefore, the expression pattern of this multigene family was investigated using RT-PCR in experimental infections and in samples of patients suffering from oral candidosis. The findings illustrate that individual *LIP*s were differentially regulated in a mouse model of systemic candidosis with some members showing sustained expression, while others were transiently expressed or even silent. It is demonstrated, that the *LIP* expression profile depended on the stage of infection rather than on the organ localization. This temporal regulation of lipase gene expression was also detected in an experimental model of oral candidosis. Furthermore, the expression of candidal lipase genes in human specimens is shown for the first time. The present study indicates, that *LIP1-10* constitute a new family of virulence factors.

380. Mating Types of *Pyrenophora teres* and *P. graminea*, the causal agents of net blotch and leaf stripe of barley.

Frank J. Maier, Gerrit Mohrmann, Maram Girgi, Bettina Wagner, and Wilhelm Schaefer. University of Hamburg, Institute of General Botany, Department of Molecular Phytopathology and Genetics (AMP III), Ohnhorststr. 18, D-22609 Hamburg, Germany.

Net bloch, caused by the leaf pathogen *Pyrenophora teres* is, one of the most devastating diseases of cultivated barley (*Hordeum vulgare*). Only moderate resistance to this disease is available in the field and nothing is known about the fungal genes that condition pathogenicity or that cause the cultural variability observed. We initiated molecular genetic studies of this pathogen to address these issues and understand its life cycle. Mating in fungi is controlled by mating type genes. The fungal class of ascomycetes is characterized by, as regards their mating behaviour, three types. 1. sterility, 2. self-incompatibility (heterothallism) and 3. self-compatibility (homothallism). In most ascomycetes, mating is controlled by a single locus, the MAT genes. The two alternate forms are not classical alleles, but rather idiomorphs, because the highly dissimilar MAT genes (MAT 1 and MAT 2) are located between highly conserved flanking sequences at the same locus. The barley pathogen *P. teres* is a heterothallic ascomycte. We cloned the responsible genes for sexual reproduction (MAT 1 and MAT 2) of different *P. teres* isolates and of the closely related *P. graminea*, causal agent of stripe disease of barley. Sequence comparisons of the different mating type genes will be presented.

381. Analysis of *AVR-Pita* Gene Function in Pathogenicity and Host Specificity. Barbara Valent[1], Melinda Dalby[1], Prasanna Kankanala[1], Darcey Klaahsen[1], Yulin Jia[2] and Gregory T. Bryan[3]. [1]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502. [2]USDA-ARS, Dale Bumpers National Rice Research Center, Stuttgart, AR, 72160-0287. [3]AgResearch Grasslands, Palmerston North, New Zealand

We continue our characterization of the rice blast *AVR-Pita* gene and its corresponding resistance gene *Pi-ta*. Sequence analysis suggests that *AVR-Pita* encodes a secreted zinc metalloprotease of the Deuterolysin metalloprotease (M35) family. Our current data suggest that the Pi-ta protein is an intracellular receptor that binds directly to the mature AVR-Pita protease inside the plant cell, initiating defense responses. Expression of*AVR-Pita* is infection-specific, and the gene is highly expressed during the colonization phase of the susceptible interaction. Current research objectives include demonstrating protease activity for AVR-Pita *in vitro*, defining its natural

substrates within the infected plant tissue, and ultimately determining the relationship between protease activity and the molecular recognition event that triggers disease resistance. AVR-Pita has properties characteristic of bacterial "effector" proteins that are delivered into the cytosol of living plant cells through a specialized type III secretion system. We have initiated studies to define when and how AVR-Pita is delivered into the plant cytosol to interact with Pi-ta. A thorough understanding of *AVR-Pita* biology will lead to strategies for identification of additional fungal effector molecules and for understanding the mechanism for delivery of critical pathogenicity factors into living plant cells.

382. Xylanolytic transcriptional activators in *Cochliobolus carbonum*. John Scott Craig, Scott Baker, Olen Yoder and Jonathan Walton. MSU-DOE Plant Research Lab, Michigan State University, East Lansing, MI. and TMRI, San Diego, CA.

Indirect evidence suggests that *Cochliobolus carbonum* penetrates maize leaves by enzymatic and not mechanical means. *C. carbonum* produces a variety of extracellular cell wall degrading enzymes (CWDEs), including xylanases, pectinases, and glucanases, which may be involved in enzymatic penetration. Disruption of *ccSNF1*, the *C. carbonum* ortholog of yeast *SNF1*, results in down-regulation of CWDE expression, reduced growth on alternate carbon sources, and attenuated leaf penetration efficiency (Tonukari*et al*., 2000, Plant Cell 12:237). To understand further the role of CWDEs in fungal pathogenesis, genes related to *XlnR*, which encodes a transcriptional activator involved in the control of expression of xylanolytic and other CWDE genes in *Aspergillus niger*(van Peij *et al*., 1998, Mol Microbiol. 27:131) were studied. Two putative *C. heterostrophus* homologues of this gene were identified in the genomic sequence database at the Torrey Mesa Research Institute. The corresponding genes were isolated from *C. carbonum* and used to create strains with targeted deletions. Experiments designed to determine the effect, if any, of *XlnR* deletion on xylanolytic gene expression and pathogenicity of *C. carbonum* are underway; results will be reported in the relevant poster session.

383. The role of the trichothecens in different isolates of *Fusarium graminearum* causing *Fusarium* head blight (FHB) in cereals. Frank J. Maier[1], Simon Henning[1], Thomas Miedaner[2], and Wilhelm Schäfer[1]. [1]University of Hamburg, Institute of General Botany, Department of Molecular Phytopathology and Genetics (AMP III), Ohnhorststr. 18, D-22609 Hamburg, Germany; [2]University of Hohenheim, State Plant Breeding Institute (720), 70593 Stuttgart, Germany.

Head blight caused by the ascomycetic fungus *Gibberella zeae* (*Fusarium graminearum*) is one of the most destructive diseases of cereals. It causes yield reductions and contaminates grain with mycotoxins, which constitutes a potential risk for human and animal nutrition. One important class of mycotoxins produced by several *Fusarium* species are the trichothecene derivates (e.g. nivalenol, deoxynivalenol). Trichothecenes accumulate in *Fusarium*-infested food and non-specifically affect most eukaryotes.We want to investigate whether virulence is only determined by the presence of the trichothecenes or is a quantitative character that is heterogeneously determined by several factors that differ from one isolate to the other. Three isolates of *F. graminearum,* well characterized in field experiments, were selected: one medium aggressive isolate that produces nivalenol, another medium aggressive isolate which forms deoxynivalenol. The third one is highly virulent and produces high levels of deoxynivalenol. The *Tri*5 genes in these three isolates were disrupted. A general biochemical and molecular biological analysis as well as the phytopathogenic properties of the mutants will be presented.

384. Plant recognition and appressoria formation in arbuscular mycorrhizal symbiosis. Natalia Requena and Esther Serrano Plant Ecophysiology Dept., University of Tübingen, Auf der Morgenstelle 1, D-72076 Tübingen.

Symbiosis formation in AM fungi is induced upon a partner recognition event that switches the developmental programme of the fungus and triggers appressorium formation. In the absence of host root recognition the fungus retracts back its protoplasm and suffers a temporary growth arrest. This developmental switch is the subject of our study. We have identified a fungal novel gene, GmGIN1, exclusively expressed during the out planta phase. The full-length GmGIN1 cDNA encodes a protein of 429 amino acids with two-domain structure and a putative self-splicing activity. The N-terminal domain shares sequence similarity to a novel family of GTP binding proteins. The C-terminus has a striking homology to the C-terminal part of hedgehog proteins from metazoa. Hedgehog proteins suffer autoproteolysis catalyzed by the carboxy terminus. The splicing reaction renders an active N-terminal domain covalently modified by a cholesterol moiety at its carboxy end. Our hypothesis is that GmGIN1 also suffers

autoproteolysis to generate the functional GTPase protein. In order to show the splicing activity of GmGIN1, 207 aa from the carboxy terminus were expressed in E. coli as an N-terminal His6 tag fusion protein. A splicing reaction involving the conserved GCF motif should render two peptides with molecular masses of 21 and 7 kDa. A small amount of spliced protein could be observed after induction of splicing activity with DTT. Further work is in progress to improve the yield of active protein and the splicing efficiency.

385. Hipervirulent strains of *Botrytis cinerea* show altered respiration. Arranz, M., Eslava, A.P., Díaz-Mínguez, J.M. and Benito, E.P. Área de Genética. Centro Hispano-Luso de Investigaciones Agrarias (CIALE). Universidad de Salamanca. 37007. Salamanca. Spain.

Botrytis cinerea is an important plant pathogenic fungus with a wide host range. In plant-fungal pathogen interactions the analysis of differential gene expression has been utilized to isolate genes specifically induced during pathogenesis. This strategy allowed us to isolate gene *Bde47*. Sequence analysis predicted mitochondrial inner membrane localization for the protein encoded by *Bde47* . Mutants altered in gene *Bde47*, obtained by gene replacement, show increassed aggressiveness on several hosts and produce higher amounts of active oxygen species, in addition to several other alterations during saprophytic growth. We propose that these phenotypes are consequence of altered mitochondrial activity. First, to confirm the mitochondrial localization of the *Bde47* gene product we have obtained transformants expressing the GFP fused to the *B. cinerea Bde47* gene. Confocal microscopy analysis demonstrated that the *Bde47* gene product is targeted to the mitochondria. Second, to investigate whether or not the hipervirulent mutant strains are altered in mitochondrial activity, we measured and compared O_2 consumption in the *B. cinerea* wild type strain and in two representative gene replacement mutant strains. The data obtained demonstrated a higher level of oxygen consumption in both mutant strains. Studies using inhibitors either of complex IV or of the alternative oxidase (Antimycin A and Salicylhydroxamic acid, respectively) indicated that the activity of complex IV is severely reduced in the mutant strains, while oxygen consumption through the alternative oxidase pathway is highly increased.

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386. Characterisation of aminophospholipid translocases during infection by the rice blast fungus *Magnaporthe grisea*. Martin J. Gilbert and Nicholas J. Talbot. School of Biological Sciences, University of Exeter, Washington Singer Laboratories, Perry Road, Exeter EX4 4QG, United Kingdom.

Plant infection by the rice blast fungus *Magnaporthe grisea* is brought about by the action of specialised infection cells called appressoria. These cells generate enormous turgor pressure, which is translated into the necessary force that allows the fungus to breach the plant cuticle. Targeted gene disruption of the *M.grisea* PDE1 gene showed its importance in penetration hypha development and pathogenicity. The predicted PDE1 gene product showed highest similarity to members of the aminophospholipid translocase group of P-type ATPase and was shown to be a functional homologue of the yeast ATPase gene ATC8. Expression studies showed that PDE1 is expressed at low levels in all stages of the *M. grisea* development. The sub-cellular localisation of PDE1(p) is being examined using a C-terminal GFP-tagging approach and also by using purified antibodies raised against the large central loop of PDE1(p). Biochemical studies are also currently underway to ascertain the substrate specificity of the PDE1(p). To further analyse the possible roles of aminophospholipid translocases during *M. grisea* infection, another member of this group MgDRS2 has been identified and its relationship to PDE1 is being investigated.

387. *PEP2* serves as a gratuitous pathogenicity gene in *Nectria haematococca*. Li-Feng Chen[1], Yinong Han[2], Haruhisa Suga[1,3] and H. Corby Kistler[1,4]. [1]University of Minnesota, St. Paul, MN 55108, USA; [2]National Research Council, Halifax, Canada; [3]Molecular Genetics Research Center, Gifu University, Gifu, Japan; [4]USDA ARS Cereal Disease Laboratory, St. Paul, USA

A gene cluster consisting of several genes that contribute to the pathogenicity of *Nectria haematococca* to pea plants is located on a fungal supernumerary chromosome. Among the cluster's pea pathogenicity (*PEP*) genes, *PEP1*, *PEP2* and *PEP5* as well as *PDA1* (the gene for pisatin demethylase) can independently increase pathogenicity to pea when added individually to an isolate that lacks the 1.6 Mb chromosome containing the *PEP* cluster. *PEP2* is a gene

encoding a 233 amino acid protein with sequence similarity to polyadenylate binding proteins. To further examine the contribution of the *PEP2* gene to fungal pathogenicity a plasmid was constructed for *PEP2* gene replacement. A 13 kb *Bam*HI / *Sal*I fragment including *PEP2* and flanking sequences was cloned in *E. coli*, and an internal 3.5 kb of *Bgl* II fragment including the entire *PEP2* gene was replaced by a fungal hygromycin resistance cassette to produce the new plasmid pUCPEP2. This plasmid was used to transform wild type strain 77-13-5. Of the several transformants obtained, two (called P4 and P5) were confirmed by Southern hybridization to be gene replacements, while four transformants (P1, P6, P7 and P8) were due to ectopic integration of pUCPEP2. Another transformant (P40) was found to have lost the entire 1.6 Mb supernumerary chromosome. Tests on pea epicotyls and roots showed there was no significant difference in pathogenicity between gene replacement and ectopic transformants but that P40 was significantly lower in pathogenicity than 77-13-5. We conclude that while *PEP2* can increase pathogenicity of a strain lacking the chromosome containing the *PEP* gene cluster, it is not essential for maximum pathogenicity in strains containing an otherwise intact 1.6 Mb chromosome. *PEP2* thus may represent a gratuitous or functionally redundant pathogenicity factor in some genetic backgrounds.

388. Analysis of the MAP kinase-signalling pathway in the mycoparasitic response of *Trichoderma atroviride*. Velázquez-Robledo Rocío, Artemio Mendoza-Mendoza, and Alfredo Herrera-Estrella. Department of Plant Genetic Engineering, Centro de Investigación y Estudios Avanzados. Unidad Irapuato. Apartado Postal 629. 36500, Irapuato Guanajuato., México.

Fungi belonging to the genus *Trichoderma* are ubiquitous microorganisms in soil. Several species of the genus have been used as biocontrol agents against a broad spectrum of phytopathogenic fungi. Parasitic response in *Trichoderma* has been well documented and is now referred as mycoparasitism. Mycoparasitic response involves the production of volatile antibiotics, rhizosphere competence and production of hydrolytic enzymes such as beta-1,3 glucanases, chitinases and proteases. In the presence of a potential host, *T. atroviride* tends to coil around the host hyphae followed by penetration due to the degradation of the outer surface of the host. Expression of a basic protease (*prb1*), and an endochitinase (*ech42*) are linked to this later event. These two hydrolytic enzymes seem to be regulated by different environmental factors. The use of specific inhibitors revealed the relevance of a MAPK signalling pathway in the induction of *prb1* and *ech42* mediated by nitrogen and carbon limitation respectively. A gene encoding a MAPKK kinase homologous to the yeast kinase Ste11 was cloned from the mycoparasite *T. atroviride* and named *tmk11*. This gene has a single copy in *Trichoderma* genome and showed high similarity to *ncr1* from *Neurospora crassa*. Tmk11 null mutants were obtained by antisense strategy. The relevance of this kinase during the interfungal relationship between *T. atroviride* and *Rhizoctonia solani* will be discussed.

389. Isolation and characterization of GCN5 histone acetyltransferase gene from *Ustilago maydis*. Juan González-Prieto, Cristina Reynaga-Peña, Angel Domínguez, and José Ruiz-Herrera. Depto. de Ingeniería Genética, CINVESTAV Unidad Irapuato, Irapuato, Mexico.

Isolation of a histone acetyltransferase gene from *Ustilago maydis* was carried out by specific hybridization to a gene fragment obtained by polymerase chain reaction. The gene (UmGCN5) contains an open reading frame of 1421 bp encoding a putative protein of 473 aminoacids. This protein exhibits a high degree of homology with histone acetyltransferases from different organisms. Null mutants were constructed by substitution of most of the coding sequence with the hygromycin B resistance cassette. Mutants displayed a slight reduction in growth rate under different conditions, grew as mycelial cells, and formed fuzz-like colonies under all conditions where wild-type strains grow yeast-like. cAMP addition failed to revert this phenotype. The most important phenotypic characteristic of the null mutants was their loss of virulence, and their incapacity to form teliospores.

390. An inducible defense mechanism against nitric oxide in *Candida albicans*. Wiriya Chiranand[1], Breanna Ullmann[1], Hadley Myers[1], Qiang Zhao[1], Paul R. Gardner[2], and Michael C. Gustin[1]. [1]Biochem & Cell Biol, Rice Univ, Houston, TX. [2]Children's Hosp Med Ctr, Cincinnati, OH

The opportunistic pathogen *Candida albicans* poses a serious threat to patients with compromised immune systems. Immune cells respond to *C. albicans* with a complex arsenal that includes production of damaging reactive oxygen and nitrogen radicals. Whether*Candida* possesses a means to defend against free radicals such as nitric oxide (NO) is yet undetermined. In the yeast *Saccharomyces cerevisiae* and various bacteria, defense against nitric oxide involves an NO-consuming flavohemoglobin, and we have identified three homologous genes in the *C. albicans*

genome. Using deletion mutant strains constructed for each gene, we demonstrated that just one homologue, *CaYHB1*, is responsible for significant nitric oxide consumption and detoxification. In contrast to nonpathogenic *S. cerevisiae*, NO consumption by *C. albicans* is greatly enhanced by exposure to nitric oxide. Correspondingly, *CaYHB1* mRNA levels are rapidly and dramatically increased by various NO-generating agents or low concentrations of NO sources but not by other oxidants. Loss of *CaYHB1* results in a higher sensitivity of *C. albicans* to NO-mediated growth inhibition. Thus *C. albicans* utilizes a rapid, specific and highly inducible NO defense mechanism involving one of three putative flavohemoglobin genes. Funding: NSF and NIH

391. Genetic characterization of Fusarium wilt of Arabidopsis. Andrew C. Diener and Frederick M. Ausubel. Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114 USA.

A wilt disease of *Arabidopsis thaliana* can be instigated by pathogenic forms of*Fusarium oxysporum*. The formae speciales, *conglutinans, raphani and matthioli*, isolated from crucifer hosts are pathogenic to Arabidopsis as well. The susceptibility is specific because Arabidopsis is completely resistant to pathogenic forms from flax and tomato. Different ecotypes (or varieties) of Arabidopsis display varying degrees of natural resistance to the crucifer pathogens. We have begun to characterize the genetic interaction between Arabidopsis and Fusarium by examining the resistance and virulence, respectively, of both plant and fungal mutants. Our progress in screening for Fusarium mutants that have defects in virulence will be detailed.

392. Genes for pea pathogenicity and a gene for utilization of a specific pea root exudate are located on the same supernumerary chromosome in *Nectria haematococca*. Marianela C. Rodriguez and Hans D. VanEtten. Department of Plant Pathology, University of Arizona

Nectria haematococca can exist in a wide range of environments, although individual isolates have a limited habitat range. Previous research indicated that habitat diversity is partially due to the presence of supernumerary chromosomes containing host specifying genes. These chromosomes have been called "Conditionally Dispensable" (CD) since they are not needed for axenic growth, but they are important in expanding the habitat of individual isolates. One of these CD chromosomes carries a cluster of genes for pea pathogenicity, called the *PEP* cluster. Preliminary work has suggested that the same CD chromosome that carries the *PEP* cluster, also carries genes for the utilization of homoserine (HS), which is a transitional amino acid present in large amounts in the root exudates of pea plants. Recent results have shown that isolates cured of the CD chromosome by benomyl treatment can not grow on HS. These results verify that genes for homoserine utilization (*HUT*) are located on the same CD chromosome as the *PEP* genes. Conventional genetics indicate that the *HUT* phenotype is encoded by more than one gene, and that at least one *HUT* gene is on a non-dispensable chromosome. We propose that *HUT* genes provide isolates carrying them a competitive advantage in the pea rhizosphere, prior to the establishment of a pathogenic association with the pea roots.

393. Saturation Mutagenesis of *Magnaporthe grisea* using DNA Insertions- a genomics approach. Sara L. Tucker[1], Yan Meng[2], Gayatri Patel[2], Natalia Kaczkurkin[1], Yong-Hwan Lee[3], Nicole Donofrio[3], M. Alejandra Mandel[1], Collin LeMaster[1], Melania Figueroa[1], Brian King[2], Love Gill[2], Jordan Brock[2], Ravi Rajagopalan[3], Douglas Brown[3], Thomas Mitchell[3], Ralph Dean[3], Mark Farman[2], Marc Orbach[1], [1]University of Arizona, Tucson, AZ 85721,[2]University of Kentucky, Lexington, KY 40546, [3]North Carolina State University, Raleigh, NC 27695 USA

Magnaporthe grisea represents a model organism for the study of fungal pathogenicity and growth. We are taking a functional genomics approach to study the infection of rice by *M. grisea*. As part of an NSF-funded project that involves collaboration between 9 research groups at 6 universities we are creating a collection of 50,000 DNA insertion lines in the*M. grisea* strain 70-15. Our goal is to disrupt all genes encoded in the *M. grisea*genome in order to determine which genes are important for pathogenicity on rice. The mutants are being generated using transforming constructs containing a Hygromycin resistance cassette flanked by promoterless fluorescent protein genes. Constructs differ in their end sequences (AT vs. GC rich ends) in an attempt to maximize genome coverage and to assess whether the ends target insertion into AT- or GC-rich regions. Standard and *Agrobacterium tumefaciens*-mediated transformation methods are being used to introduce the DNA into*M. grisea*. All 50,000 insertion lines will be analyzed for defects in pathogenicity, metabolism and alterations in morphology. Such a project necessitates development of high throughput methods for the generation and screening of putative

transformants. In addition, a database has been created for recording all of the data generated per transformant. The advances made so far and the optimization of techniques for genetic manipulation of this fungus will be discussed.

394. Identification of pathogenicity determinants in the interaction between the oomycete plant pathogen *Peronospora parasitica* and Arabidopsis. Jim Beynon, Rebecca Allen, Peter Bittner-Eddy, Laura Grenville and Anne Rehmany. Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK.

Peronospora parasitica is the causal agent of downy mildew on Arabidopsis and Brassica crops. We have cloned the Arabidopsis resistance genes *RPP13* and*RPP1* that recognise the downy mildew isolates Maks9 and Emoy2, respectively. These two interactions result in very different resistance phenotypes. *RPP13* elicits a localised necrotic lesion whereas *RPP1* results in a spreading necrotic lesion. In order to study the basis of these different interaction phenotypes we are cloning the corresponding avirulence genes, *ATR13* and *ATR1*. We have used a map based cloning approach and located the genes to overlapping BAC contigs. The mapping cross has been shown to be segregating for up to 15 different avirulence genes. Using Suppression Subtractive Hybridisation we have identified a range of genes that are specifically up-regulated on infection of Arabidopsis. An analysis of their structure and their relationship to the avirulence genes will be presented.

395. Negative regulation of *prb1* and *ech42* from *Trichoderma atroviride* is driven by physical contact with the host. Carlos Cortes[1,2], Vianey Olmedo-Monfil[1], Alfredo Herrera-Estrella[1]. [1]Plant Genetic Engineering Department. CINVESTAV-IPN. Irapuato, Guanajuato. Mexico. [2]Escuela de Quimico-Farmacobiologia. UMSNH. Morelia, Michoacan. Mexico.

Interfungal relationships depend on recognition between the associated species.*Trichoderma atroviride* is able to establish necrotrophic associations with a wide range of fungi, most of them classified as phytopatogens. This ability has been successfully used in the biocontrol of several fungal pests. It has been proposed that the mycoparasitic activity in *T. atroviride* has limited specificity based on the fact that several fungal species are suceptible to *Trichoderma* attack. In this regard, the induction of hydrolytic enzymes play a key role in completing the parasitic phenomenum. Prb1 and Ech42 are normally induced when*Trichoderma* is directly confrontated with a potential host even if physical contact is avoided. When *Sclerotium rolfsii* was used as a host the induction of *prb1* and*ech42* was only detected when both host and parasite were physically separated by a cellophane membrane. On the contrary, transcriptional repression of both genes occurred when physical contact took place. *Trichoderma virens*, a closely related specie, showed induction of the homologous genes to *prb1* and *ech42* when confrontation was carried out with *S. rolfsii* demostrating a specie-specific response. Fusion of the *prb1* promoter to the *gfp* reporter gene allowed the detection of the possible negative regulatory element.

396. New transformation system for the human pathogenic yeast, *Candida parapsilosis*, based on FLP-mediated site specific recombination. Attila Gacser[1], Siegfried Salomon[1], Joachim Morschhaeuser[2] and Wilhelm Schaefer[1]. [1]University of Hamburg, Institute of General Botany, Department of Molecular Phytopathology and Genetics (AMP III), Ohnhorststr. 18, D-22609 Hamburg, Germany. [2]University of Wuerzburg, Institute for Molecular Biology of Infectious Diseases, Röntgenring 11, D-97070 Wuerzburg, Germany

The opportunistic human pathogen *Candida parapsilosis* causes superficial cutaneous infections and systemic candidosis. Here, we describe an efficient system for gene disruption in this fungus, using direct transformation and dominant selection of a clinical isolate. This omits the necessity to create auxotrophic mutants. The transformation system is based on the MPA^R (mycophenolic acid) resistance marker and its subsequent deletion by FLP-recombinase mediated, site specific recombination. The MPA^R cassette was used to generate *C. parapsilosis* mutants disrupted for one allele of either the lipase1 gene or lipase2 gene. This flipper system for genetic manipulations enhances the efficiency of molecular biological research in *C. parapsilosis.* Thus, it helps to improve our knowledge of its biology and virulence.

397. Downy Mildew Genomics and Plant Disease Resistance. Posthuma, K., Elberse, J., Weisbeek, P. and van den Ackerveken, G. Molecular Genetics. Utrecht University, Utrecht, The Netherlands.

Downy mildews infect many important crops grown in temperate climates. To protect crops from downy mildew disease, plant breeders have crossed in natural resistance genes. However, resistance is usually rapidly overcome by the pathogen. This project aims to identify new resistance genes that mediate the recognition of important pathogen proteins and may therefore be more durable. A genomics approach is used to identify downy mildew genes that encode secreted proteins and that are specifically expressed during the infection process. On the one hand, these genes can be used by plant breeders to select potential breeding lines. On the other hand, this project will enable us to study which downy mildew genes are required in the infection process. Two downy mildew – plant interactions are studied: *Peronospora parasitica* – Arabidopsis and *Bremia lactucae* – lettuce. Complementary DNA libraries of spores and downy mildew-infected plants have been constructed to collect a large number of Expressed Sequence Tags (ESTs). These ESTs will be screened for signal peptides and for similarity to genes or proteins in the public databases. Microarray technology will be used to study the expression of these genes during infection of the host. *B. lactucae* genes encoding extracellular proteins will be transiently expressed in lettuce, and lines reacting with a hypersensitive response will be tested further for downy mildew resistance. The selected lines used by lettuce breeders to obtain more durable resistance to downy mildew disease.

398. Stage-specific gene transcription in *Phytophthora infestans* prior to and during the early stages of potato infection. Anna O. Avrova, Eduard Venter*, Paul R. J. Birch, and Stephen C. Whisson. Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, U.K.*Department of Genetics, University of Pretoria, Pretoria 0002, South Africa.

Phytophthora infestans, the oomycete agent of potato and tomato late blight, produces several different cell types prior to and during the early stages of potato infection. All of these cell types can be easily generated and studied in the absence of the host plant and so form the basis for developmental stage-specific gene discovery. We have used amplified fragment length polymorphism (AFLP)-based mRNA fingerprinting (cDNA-AFLP) and suppression subtractive hybridisation (SSH) to identify transcripts that were up-regulated in germinating zoospore cysts, and germinating zoospore cysts with appressoria, but not in vegetative mycelium. These transcripts included representatives of most major classes of heat shock proteins:*hsp60, hsp70, hsp90* and *hsp100*. Other stress-related, and potential pathogenicity genes were also identified. Real-time RT-PCR was used to quantify the expression of selected transcripts originating from germinating cysts, with and without appressoria, relative to the constitutively expressed *actB* gene, in vegetative mycelium, germinating cysts, and at three time points post-inoculation of potato cultivar Bintje (15, 48, and 72 hours). All of the transcripts were up-regulated in germinating cysts, and many were found also to be up-regulated*in planta*.

399. Cloning avirulence genes from *Phytophthora infestans*. Miles Armstrong, Anna Avrova, Steve Whisson, Paul Birch. Plant Pathogen Interactions Scottish Crop Research Institute.

Phytophthora infestans is a hemi-biotrophic pathogen that exhibits gene-for-gene interactions with its host potato. Compared to bacterial pathogens, relatively few fungal avirulence genes have been cloned and only one from the oomycetes. We aim to clone and characterise avirulence genes from the oomycete pathogen *P. infestans* as a first step in studying the molecular basis of host/oomycete recognition and specificity. Two approaches are being followed: The Avr2 gene is being mapped in a segregating population of F1 individuals derived from a sexual cross. Secondly, a number of candidate genes identified in silico from ESTs are being amplified and sequenced from isolates in order to identify SNPs associated with specific avirulences. This approach has identified a strong candidate for the Avr3 gene.

400. *Agrobacterium tumefaciens*-mediated genetic transformation of the phytopathogenic oomycete *Phytophtora palmivora*. Hoang Quoc-Khanh and Tran Hoang Ngoc Ai. Institute of Tropical Biology 1 Mac Dinh Chi St., Dist. 1, Ho Chi Minh City, VN-70000 VIETNAM

Agrobacterium tumefaciens-mediated transformation has been successfully applied to the phytopathogenic fungus *Phytophtora palmivora*. The transformants were resistance to hygromycin B as the selective trait, governed by *Aspergillus nidulans* gpd promoter and*A. nidulans trpC* terminator. The transformants appeared to be stable through mitotic and meiotic cell division. The presence of the hph gene was detected by PCR. Our findings indicate that the technique offers potential as an alternative tool to conventional transformation techniques and should address to the *P. palmivora* pathogenicity and decision-makes to disease management.

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401. Efficient production by *Aspergillus awamori* of a Llama antibody fragment fused to a peroxidase. B. Christien Lokman[1], Vivi Joosten[1], Marc Roelofs[1], Niels van den Dries[1], Robin J. Gouka[2], C. Theo Verrips[2], and Cees A.M.J.J. van den Hondel[1]. [1]TNO Nutrition and Food Research, P.O. Box 360, 3700 AJ Zeist, the Netherlands. [2]Unilever Research Vlaardingen, P.O. Box 114, 3130 AC Vlaardingen, the Netherlands

The development of fusion proteins consisting of antibody fragments and enzymes is of great medical and industrial importance. Previously, our group has demonstrated that single chain Fv antibody fragments (scFv) could be efficiently produced in *Aspergillus awamori* (Frenken et al., 1998). Recently, we have studied the production of Llama heavy-chain antibody fragments (VHH) by *A. awamori*. The advantage of VHH over ScFv antibody fragments is that VHHs are devoid of light chains (Figure 1). Furthermore, VHH lack the hydrophobic regions that are normally facing the variable domain of the light chain and are therefore suggested to be better secreted than scFv fragments. A suitable enzyme for industrial applications is *Arthromyces ramosus* peroxidase (ARP). This 41 kD monomeric glycoprotein has a broad specificity for phenolic and anilinic hydrogen donors. In previous studies we have demonstrated that there is no heme limitation during overproduction of ARP in *A. awamori*. Under control of the endoxylanase promoter secretion of active ARP was achieved up to 0.8 g/L in shake flask cultures. Fusions between enzymes and VHHs permit interesting applications due to the fact that VHHs direct the enzymes to the place where they should act. As an example we studied the integrity of N-terminal and C-terminal fusions of ARP with a VHH fragment against the azo-dye RR6 after production in *A. awamori*.

402. Identification of Manganese-inducible genes in *Aspergillus niger* using suppression subtractive hybridization. Ziyu Dai, Jon Magnuson and Linda Lasure. Processing Science, Pacific Northwest National Laboratory, Richland WA.

Morphology of filamentous fungi in fermentation processes is critical to optimal product output. The proper morphology of citric acid production strains of *Aspergillus niger* is regulated by manganese (Mn^{2+}) and other factors. Detailed examination of the effects of Mn^{2+} on *A. niger* morphology formation shows that filamentous hyphae of *A. niger* developed rapidly at Mn^{2+} concentrations of 14 ppb or higher, while citric acid production dramatically decreased. Molecular mechanisms involved in Mn^{2+} induction of morphology formation in *A. niger* have not been well defined. Here, suppression subtractive hybridization identified fifteen genes differentially induced when *A. niger* grew at 1000 ppb Mn^{2+} and seven genes differentially expressed at 10 ppb Mn^{2+}. Among the fifteen filamentous form associated genes, nine are unknown and six have some degree of identity to genes in other organisms. Similarly, six non-filamentous form associated genes are unknown, but one clone, Arsa-1, exhibits homology to a yeast gene. Elevated mRNA levels in ten filamentous form associated genes were observed 40 and 120 min after the addition of 1000 ppb Mn^{2+} to 12 hr cultures, while the mRNA of all seven non-filamentous form associated genes were rapidly degraded. Transcription of the other five filamentous form associated genes were dramatically increased during the first 40 min following Mn^{2+} induction and thereafter rapidly degraded. These findings create the opportunity for detailed studies on genes and enzymes involved in morphology determination. These results also reveal that SSH is a powerful and sensitive technique for the detection of differential gene expression in *A. niger* morphology formation.

403. Global Expression Profiling of the Lignin Degrading Fungus *Ceriporiopsis subvermispora* for the Discovery of Novel Enzymes. Debbie S. Yaver, Barbara Weber and Jeff Murrell. Novozymes Biotech, Davis, CA.

The unique ability of white rot fungi to degrade all components of wood, including lignin, has attracted considerable biotech interest for several decades. *Ceriporiopsis subvermispora* is among the most selective lignin-degraders. Pretreatment of wood chips with *C. subvermispora* prior to mechanical pulping has been shown to reduce energy consumption by 30-40%. Using DNA microarray technology, global gene expression profiling of *C. subvermispora* was used to discover novel enzymes whose expression is induced during growth on mechanical pulp. Plasmid DNA was isolated from 50,000 independent clones of a random genomic library containing 2 to 3 kb genomic fragments and spotted on poly-lysine coated glass slides at a density of 10,000 per slide. Hybridization probes were prepared from RNA which was isolated from thirty day old cultures grown on either minimal medium or hardwood mechanical pulp labeling them with either Cy-3 or Cy-5 dyes. Hybridization to the first 20,000 clones identified 20

clones whose expression is induced due to growth on pulp. Sequence analysis has shown that the 20 clones actually represent 11 unique clones. One of these clones has significant homology to manganese peroxidases from white rot fungi including those previously identified from *C. subvermispora*; however, it is novel. We will report the heterologous expression and characterization of the peroxidase as well as the characterization of the other pulp-induced clones.

404. Targeted Illegitimate Recombination in *A. nidulans*. R. David Laidlaw and Jay D. Keasling. Department of Chemical Engineering, University of California at Berkeley, Berkeley CA, USA.

Metabolic engineering of fungal species often suffers from low transformation efficiencies, a low number of selectable markers, difficulty controlling insertion sites for transformed fragments and the possibility that DNA will insert into multiple ectopic locations. Assembling heterologous pathways into stable isogenic production strains presents a plethora of technical challenges. Elegant systems based on traditional homologous recombination have been developed to simplify insertion or disruption of gene-cassettes in filamentous fungal hosts such as *N. crassa* and *A. nidulans*. Few possibilities currently exist for single-step insertion of large multi-gene pathways to a single location within the genome. Here, we introduce a technique we have named 'Targeted Illegitimate Recombination' as a strategy for inserting DNA into the genome independent of a need for homology. We have develloped a modified REMI approach to insert DNA fragments at an engineered genomic site within *A. nidulans*. To reduce the uncontrolled nature by which REMI insertions typically occur, we have spliced a silent recognition sequence for the commercially-available homing endonuclease*I-SceI* into a selectable marker gene. We present results demonstrating the usefulness of this technique for inserting DNA fragments into *A. nidulans* as the first step towards a suite of possibilities for metabolic engineering tools. Funding for this project provided by Merck and Co.

405. Modification of the N-glycosylation pathway of lower eukaryotes to a mammalian type. Vervecken, W., Callewaert, N., Geysens, S. and Contreras, R. Fundamental and Applied Molecular Biology, Ghent University and Flanders Interuniversity Institute for Biotechnology, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium.

Lower eukaryotes only synthesise N-glycans of the high-mannose type, whereas human glycoproteins have a very divers, complex type of N-glycans. Redirection of the fungal pathway, even to a simple hybrid or complex mammalian type requires several genetic interventions such as gene knock-outs and heterologous expression of mammalian glycosyl transferases. Furthermore, additional in vitro enzymatic manipulations may be required. In general, it seems that humanising N-glycans from filamentous fungi is an easier task than modifying yeast protein linked carbohydrates because the long alfa-1,6-arm apparently is absent. The problem is reduced to importing an efficient alfa-1,2-mannosidase and addition of complex sugar glycosyl transferases. We started a strategy to humanise the N-glycosylation pathway in the filamentous fungus Aspergillus niger NW195. In a first step a HDEL tagged a-1,2-mannosidase from *Trichoderma reesei* was introduced. The over expression of this enzyme lead to the conversion of the majority of the N-glycans to Man5GlcNAc2 (ca. 80%). In a second step the over expression of human Nacetylglucosaminyltransferase I lead to the detection of GlcNAcMan5GlcNAc2 structures. The percentage conversion of Man5GlcNAc2 to GlcNAcMan5GlcNAc2 was inversely related to the amount of protein that was synthesised, ranging from ca. 40% to merely all. Ultimately, the terminal sialic acid should be added using in vitro procedures.

406. Genomics of *Fusarium venenatum*: An Alternative Fungal Host for Making Enzymes. Randy M. Berka, Beth A.Nelson, Elizabeth J. Zaretsky, Wendy T. Yoder, and Michael W. Rey. Novozymes Biotech, Davis, CA.

Fusarium venenatum A3/5 (formerly *F. graminearum* Schwabe A3/5) has been used since 1985 as the commercial source of Quorn™ mycoprotein, a processed form of fungal mycelia applied in several human food products to simulate chunks of chicken or beef. Regulatory approval of the organism for human consumption made it an attractive candidate to consider as a host for the production of industrial and food grade enzymes. Systems for genetic manipulation and transformation of *F. venenatum* cells have been developed together with several strong promoters and selectable markers for the introduction and expression of heterologous genes. Recent marketing of a heterologous xylanase and a fungal trypsin have provided a "proof of concept" for *F. venenatum* as a useful alternative to more traditional fungal hosts such as *Aspergillus niger* or *A. oryzae*. However, compared to the latter organisms and well-studied model fungi such as *Neurospora crassa* and *A. nidulans*, information regarding the genomics of *F. venenatum* is inadequate. This poster provides one of the first overviews of *F. venenatum* genomic

information based on a compilation of expressed sequence tags and chromosomal gene sequences to initiate momentum for more comprehensive genome sequencing efforts.

407. Expression of laccase gene from *Coriolus versicolor* in transgenic tabacco for remediation. Yosuke Iimura[1], Seiichirou Ikeda[2], Tomonori sonoki[2], Shinya Kajita[2], Kenji Tatsumi[1], and Yoshihiro Katayama[2]. [1]National Institute of Advanced Industrial Science & Technology, Ibaraki, Japan. [2]Tokyo University of Agriculuture & Technology, Tokyo, 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 laccase gene from *Coriolus versicolor* .

408. The influence of AmyR on extracellular enzyme production by *Aspergillus niger* is NOT limited to enzymes involved in starch degradation. P.A. vanKuyk[1], J. Visser[1], A.M. Levin[2], H.A.B. Wösten[2], Ronald P. de Vries[1,2]. [1]MGIM, Wageningen University, Wageningen, The Netherlands; [2]Microbiology, Utrecht University, Utrecht, The Netherlands

The role of AmyR in the regulation of starch degradation has been studied in detail in *A. oryzae* and *A. nidulans*. AmyR regulates genes encoding glucoamylase, alpha-amylase and alpha-glucosidase resulting in production and secretion of the enzymes. *A. niger wild type, and amyR* disruptant and multicopy strains were compared for gene expression, protein secretion, extracellular enzyme activity, morphology and growth. Production of starch degrading enzymes was strongly reduced in the *amyR* disruptant and increased in the *amyR* multicopy strains. Surprisingly, production of some extracellular enzymes, not involved in starch degradation(e.g. beta-galactosidase) is influenced in a similar manner. This was confirmed by protein secretion profiles. In addition, the expression of sugar transporter encoding genes was influenced by AmyR. No strain differences were observed with respect to growth and morphology during growth on D-fructose, D-xylose, or glycerol. However, on starch, maltose, cellulose and lactose growth was strongly reduced for the *amyR* knockout, while the *amyR* multicopies showed enhanced growth, but reduced sporulation. This indicates that AmyR not only affects production of starch degrading enzymes, but also of other extracellular enzymes involved in hydrolysis of other oligo- and polysaccharides.

409. *Aspergillus sojae*, a new system for efficient protein production. Margreet Heerikhuisen, Anneke Drint-Kuyvenhoven, Alwin Albers, Nick van Biezen, Cees van den Hondel, Peter Punt. TNO Nutrition and Food Research, Department of Applied Microbiology and Gene Technology, Utrechtseweg 48, Zeist 3704 HE, The Netherlands.

Aspergillus species are known as high-level expression hosts for the production of enzymes or metabolites. In the last two decades for the industrially used species for*Aspergillus niger, A. foetidus, A. tubigensis,* and *A. oryzae* expression systems have been developed. We describe the development of an expression system based on the koji mold *Aspergillus sojae* for the production of homologous and heterologous proteins. Transformation based on auxotrophic (*pyrG, niaD*) and/or dominant markers (*amdS*), was used to introduce the genes of interest. To improve the yield of produced protein, *Aspergillus sojae* mutants with lower protease activity, either by gene disruption or by UV mutagenesis, were isolated. To improve the fermentation yields of *Aspergillus sojae* also morphological and so-called fermentor adapted mutants with lower viscosity, were isolated. As one of the examples for heterologous protein production the production of human interleukin 6 was used. References: Heerikhuisen, M. et al. (2001) World Patent Application WO 01/09352

410. Optimization of the *Agrobacterium*-mediated Fruiting Body Tissue Method for the Transformation of *Agaricus bisporus*. Michelle Stone,[1] Carl Schlagnhaufer,[1] Lori A. MacDonald,[2] Mark P. Wach,[2] and C. Peter Romaine.[1] [1]Department of Plant Pathology, Penn State University, University Park, PA and [2]Sylvan Inc., Kittanning, PA.

We previously described a highly effective and convenient transformation system for the button mushroom, *Agaricus bisporus*, involving *Agrobacterium*-mediated delivery of DNA to fruiting body tissue (Chen et al. 2000. Appl. Environ. Microbiol. 66:4510). Herein, we have explored several parameters of the method, which when optimized have provided upwards of 100% transformation efficiency (TE) as measured by fraction of the fruiting body tissue pieces expressing a hygromycin resistance (*hph*) selectable marker gene. Experiments were conducted using an *A. tumefaciens* strain (AGL-1) carrying a binary plasmid vector (pBHg) containing the *hph* gene controlled by the *A. bisporus* promoter for the glyceraldehyde 3-phosphate dehydrogenase gene. In two independent research laboratories, reproducibly high TEs in the range of 30-100% were obtained with (1) induction of the bacterium for 2 to 24 hr at 20-25 C, and (2) co-cultivation of the bacterium and fruiting body tissue for 3 to 4 days at 18-26 C. Further, there was a trend towards higher rates of transformation using gill tissue (73% TE) as opposed to the spongy tissue derived from the caps and stems (44% TE) of fruiting bodies. Comparably high efficiencies were observed using either gill or spongy tissue from a sporeless mushroom strain (~90% TE) and either undeveloped or developed gill tissue of several commercial hybrid strains (~70% TE). The described method represents a facile tool for introducing genes into *A. bisporus* and may well be applicable to other fungi bearing fleshy fruiting bodies.

411. A Metabolic Engineering Tool for the Filamentous Fungus *Aspergillus nidulans*. Guang Yi Wang and Jay D. Keasling. Department of Chemical Engineering, University of California at Berkeley, Berkeley CA, USA.

Filamentous fungi are a prolific source of secondary metabolites. This feature along with their physiological and metabolic diversity and available fermentation protocols makes fungi a promising metabolic engineering host for the production of valuable pharmaceutical agents from fungi and other eukaryotic organisms. Nevertheless, metabolic engineering of filamentous fungi often requires introduction of multi-gene pathways under the control of fungal regulatory elements into auxotrophic strains. On the other hand, increases in numbers of genes and regulatory elements in the transformation vector always enhance the high efficiency of ectopic integrations and greatly decrease the targeted homologous recombination and transformation efficiency in DNA-mediated transformation. To overcome these obstacles, we have adapted a cosmid mediated transformation approach (NAR, 28:E97) to introduce multiple isoprenoid-gene pathways into the fungal genome. The comsid carrying the multiple isoprenoid genes was engineered with the *A. nidulans* cosmid carrying ArgB gene and the engineered isoprenoid gene expression cassettes using the Red system developed in *E. coli* (PNAS, 97:6640-6645). We present results of using this approach to introduce isoprenoid-gene pathways into *A. nidulans* for the production of isoprenoids.

412. Cloning and Expression of Glycosyl Hydrolase Cel7 family members. Paulien Neefe[1], Vicky Huynh[2], Peter Gualfetti[2] and Frits Goedegebuur[1]. [1] Genencor International BV, Archimedesweg 30, 2333CN Leiden, the Netherlands. [2] Genencor International Inc., 925 Page Mill Road, Palo Alto, Ca 94304, USA

The enzymatic degradation of cellulosic biomass to glucose is an important step for utilizing renewable resources for the creation of bioproducts. This process is performed in nature by a complex mixture of cellulolytic enzymes. Within the cellulase mixture produced by *Trichoderma reesei*, CBHI (*Hypocrea jecorina* Cel7A) is ~50% of the secreted protein and is an essential enzyme for cellulose degradation. To improve this molecule, a program has been started to identify mutants with changes in activity and in thermal stability. In addition, many previously identified CBHI homologs have been cloned, sequenced and expressed in a heterologous host. Those Cel7 proteins that were successfully expressed were purified and characterized in terms of their stability and activity.

413. Cloning of the *Chrysosporium lucknowense* CBH I (Cel7A) gene and characterisation of the encoded enzyme. Alexander V. GUSAKOV*, Tatyana N. SALANOVICH*, Fedor E. BUKHTOJAROV*, Alexander V. MARKOV*, Boris B. USTINOV*, Cora van ZEIJL**Peter PUNT**, Richard BURLINGAME† and Arkady P. SINITSYN*. *Department of Chemistry, M. V. Lomonosov Moscow State University, Moscow 119899, Russia, **TNO Nutrition and Food Research, P. O. Box 360, 3700 AJ Zeist, The Netherlands, †Dyadic International, Inc., 140 Intracoastal Pointe Drive, Suite 404, Jupiter, Florida 33477-5094, USA

Chrysosporium lucknowense, an ascomycetous cellulolytic fungus, is currently used for the commercial production of hemi(cellulases) and is being developed as a system for the expression of heterologous genes and gene discovery by high-throughput functional expression. Sequence analysis of peptides isolated from the major cellulase secreted by *C. lucknowense* showed similarity to published cellobiohydrolases. PCR amplification with primers based on these peptides resulted in the isolation of a DNA fragment homologous to cellobiohydrolases belonging to family 7

glycoside hydrolases, which includes the well-studied CBH I of *Trichoderma reesei*. The PCR fragment was used for the isolation of the corresponding gene, which we designated cbh1. The primary amino acid sequence of the *C. lucknowense* CBH I protein was deduced from the gene sequence. Two forms of *C .lucknowense* CBH I (Cel7A) were purified from the culture filtrate (52 kD and 65 kD). Their content makes up about 20% of the total extracellular protein. Analysis of the enzymatic properties of the two proteins showed that the 52 kD enzyme displayed much lower Avicel and cotton hydrolysis rates than the 65 kD enzyme, indicating the absence of a cellulose binding domain in the 52 kD protein. The thermostability of the 65 kD enzyme was significantly higher than that of the 52 kD protein, and better than that of the intact CBH I isolated from a commercial *T. reesei* preparation. Development of a genetic system for *C. lucknowense* allows selective overexpression of the *C. lucknowense cbh1* and other cellulase genes.

414. Gene cloning and characterization of a novel glutaminase from *Aspergillus sojae*. Kotaro Ito[1], Kenichiro Matsushima[1], Genryou Umitsuki[2], Yasuji Koyama[1]. [1]Research and Development Division, Kikkoman Corporation, [2]Noda Institute for Scientific Research

Glutaminase is an enzyme that catalyzes the hydrolysis of L-glutamine to L-glutamatic acid. Glutaminase plays an important role to enhance umami taste in fermented food production. Using EST information of *Aspergillus oryzae*, we cloned a novel glutaminase-encoding gene, *AsgahA*, from *Aspergillus sojae* which was similar to a salt-tolerant, thermostable glutaminase of *Cryptococcus nodaensis*. The structural gene was 1929 bp long with no intron. This glutaminase protein, AsGahA, had an amidase motif, and showed 36% homology to that of *C. nodaensis*. Introduction of multiple copies of *AsgahA* into*A. oryzae* RIB40 resulted in over expression of glutaminase activity. AsGahA was located at cell surface. AsGahA was subsequently purified from over expressing strain, and characterized. The molecular mass was estimated as 67 kDa by SDS-PAGE while it was estimated as 135kDa by gel filtration chromatography, which indicated that native form of AsGahA was a dimer. Its pH optimum was 9.5 and its temperature optimum was 50 degrees C. Analysis of substrate specificity revealed that AsGahA catalyzed not only L-glutamine but also L-asparagine, and was revealed as a glutaminase-asparaginase.

415. *Agrobacterium tumefaciens*-mediated transformation: an efficient tool for gene replacement in *Aspergillus awamori.* C.B. Michielse, A.H.A. van Dijk, M. Arentshorst, A.F.J. Ram, 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

Gene disruption or gene replacement is often used to generate precise deletion mutants in order to assess a possible function to the deleted gene. *A. tumefaciens*, a plant pathogen, which is being used for the transformation of plants, yeast and filamentous fungi, was shown to be an efficient tool for gene targeting in *Kluveromyces lactis* (Bundock et al., 1999). To determine the efficiency of gene replacement in *Agrobacterium*-mediated transformation of *A. awamori*, a systematic study was performed. The hygromycin selection marker was flanked with promoter and terminator sequence homologous to *pyrG* of varies sizes (1000 to 50 bp). Homologous recombination frequencies were determined and compared to frequencies obtained with the PEG/CaCl2 transformation method. Homologous recombination frequencies with the *Agrobacterium*-system increased 6-fold compared to the conventional method with 1000 bp flanks (30% versus 5%, respectively). Shortening the flanks to 500 and 250 bp led to a decrease in recombination frequencies to 5 and 1%, but these frequencies were again higher compared to the conventional method. By altering the length of the left and right flanking regions, it was shown that a long left flanking region increases the percentage of homologous recombination. Based on these data it can be concluded that *Agrobacterium*-mediated transformation is an efficient tool for gene replacement and that the left border of the T-DNA plays an important role in homology search and might serve as a starting point for integration. Bundock et al. (1999), T-DNA from *Agrobacterium tumefaciens* as an efficient tool for gene targeting in *Kluyveromyces lactis*, MGG 261(1): 115-21.

416. A molecular approach for the development of a biosensor using *Aspergillus nidulans*. Milton Roque and Reinhard Fischer, Philipps-University of Marburg, Dept. of Microbiology and Max-Planck-Institute for Terrestrial Microbiology, Karl- von-Frisch-Str., D-35043 Marburg, Germany

The sensitive detection of heavy metals or pesticides in soil is a challenge for the development of biosensors. We are aiming to use the soil microorganism *Aspergillus nidulans* for this purpose. The strategy will be to isolate genes,

which are highly expressed upon exposure of the living organism to the toxic compounds. The corresponding genes will be used to establish a reporter with the green fluorescent protein (GFP) as a tool. Genes which respond to general stress conditions can be used for a first evaluation of the contamination of a given soil and specific genes for a subsequent quantitative analysis. In order to develop a sensory system for general stress conditions, we have chosen superoxide dismutase (SOD). Northern blot analysis showed that *sod*-transcription is strongly induced in the presence of the herbicide diuron (100 ug/ml) and the primary metabolite 3,4 dichloroaniline (100 ug/ml), as well as the insecticide carbofuran (75 ug/ml) and the heavy metals cadmium (0.01, 0.2 and 1.0 ug/ml), mercury (2.5 ug/ml) and lead (5.0 ug/ml). In addition to transcript analyses, we fused 1.8 kb of the putative promoter region with GFP and introduced the construct in *A. nidulans*. In addition to the targeted approach, we constructed a differential library to isolate genes specifically induced upon diuron application. We used the SSH technique. We obtained fragments of genes with homology to glycosyltransferase, acetyl coenzyme A synthetase or multi drug resistance (MDR). The putative promoter region (~ 1.8 kb) from the MDR gene was transcriptionally fused to GFP. First results using the two reporter systems (SOD and MDR) will be presented.

417. Functional Analyses of Two Genes Encoding Catalytic Subunits of cAMP-Dependent Protein Kinase A (Pka) in *Sclerotinia sclerotiorum*. Wayne M. Jurick II[1], Martin B. Dickman[2], and Jeffrey A. Rollins[1] . [1]Department of Plant Pathology, University of Florida-Gainesville. [2]Department of Plant Pathology, University of Nebraska-Lincoln

Cyclic AMP-dependent protein kinase A (Pka) is involved in regulating diverse biological processes in humans, mice, yeast, and filamentous fungi. Pka mutants in phytopathogenic fungi have been shown to affect appresorial formation, pathogenicity, and mycelial growth. Results of a previous study in *Sclerotinia sclerotiorum* indicated that compounds which increase intracellular levels of cAMP inhibited sclerotial formation and increased oxalic acid production. Pka was hypothesized to be involved in mediating these effects. To test this hypothesis, a genomic clone corresponding to the catalytic subunit of Pka (*pkaS*) was isolated and disrupted between subdomains II and III using a hygromycin cassette. Southern hybridization revealed that *pkaS* was a single copy gene and targeted disruption was confirmed by PCR and genomic DNA hybridization. Interestingly, *pkaS* mutants showed no morphological aberrations in sclerotial development, were pathogenic, and produced sclerotia in the presence of cAMP-amended medium. Multiple *pka* catalytic subunit genes have been characterized in *Ustilago maydis* (*uka1* & *adr1*) and indentified in recently sequenced genomes of *Neurospora crassa* & *Magnaporthe grisea*. It is hypothesized that a additional *pka* gene encoding an additional catalytic subunit exists in *S. sclerotiorum*. Our current objective is to clone, disrupt and phenotypically evaluate a additional gene encoding a Pka catalytic subunit in *S. sclerotiorum*.

418. The *Pc-mco1* gene of *Phanerochaete chrysosporium* encodes for a novel extracellular multicopper oxidase with ferroxidase activity. Luis F. Larrondo[1*], Loreto Salas[1], Francisco Melo[1], Dan Cullen[2], and Rafael Vicuña[1]. [1]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile and Millenium Institute for Fundamental and Applied Biology. [2]USDA Forest Products Laboratory, Madison, Wisconsin 53705, USA

Lignin degradation by the white rot basidiomycete *P. chrysosporium* involves an array of extracellular oxidative enzymes, including lignin peroxidase, manganese peroxide and glyoxal oxidase. Recent studies suggest that laccases may also be produced, although this has been a controversial issue. A search of the *P. chrysosporium* genome database revealed four sequences distantly related to laccases and ferroxidases (Fet3). One of these multicopper oxidase sequences, *Pc-mco1*, is interrupted by 19 introns and is transcribed in defined media and in colonized wood. The cDNA is predicted to encode a mature MCO protein of 543 aa with a 16 aa secretion signal. Structural analysis and multiple alignments led to the identification of residues common to both laccases and Fet3. The recombinant MCO, expressed in *Aspergillus nidulans*, has a molecular weight of 78 kDa and the UV-visible spectrum confirms the presence of a copper I type center. The purified enzyme oxidizes a variety of compounds such as ABTS and aromatic amines, although it poorly oxidizes phenols. Its best substrate is Fe^{2+} (Km ~ 2 uM). Because only ceruloplasmin and Fet3 are known to oxidize Fe^{2+}, we also searched the *P. chrysosporium* database for Fet3-like sequences. The *P. chrysosporium* Fet3 gene and corresponding cDNA were cloned and characterized. This is the first report of a fungal extracellular MCO capable of oxidizing Fe, but distinct from Fet3. Our results also firmly establish the view that *P. chrysosporium* lacks conventional laccase. This work was financed by grants 8990004 and 2000076 from FONDECYT-Chile, by the MIFAB-Chile, and by the U.S. Dept. of Energy grant DE-FG02-87ER13712. * Fellow from Fundacion Andes.

419. Fungal Genomics and Tools. Marco van den Berg, Remco Klasen, Herman Pel, Han de Winde and Roel Bovenberg DSM Life Science Products, DSM Gist (624-0270), PO Box 425, 2600 AK, Delft, The Netherlands

Fungi as *Pencillium chrysogenum*, *Saccharomyces cerevisiae* and *Aspergillus niger* are the most important fungi used for a the production of a range of products within DSM Life Science Products. In order to rationalize the ongoing improvement of production strains and processes, as well as to speed up the identification of potential new products, genome seqeunces and tools are essential to embarke onto 'omics'-type of R&D. Analyzing the genomic sequences of our favourite fungi resulted not only in a wealth of new genes, but more importantly it resulted in much more insight in the organisms themselves. To be able to study these, several new and old tools were adapted tofunction in the easy 'fungus' *Saccharomyces*, as well as in the more difficult fungus *Penicillium*. Within the penicillin gene cluster of *Pencillium chrysogenum* unexpected genes were found to be amplified in higher producing strains. Using a combination of molecular and biochemical technologies we identified and demonstrated the function of several proteins in relation to penicillin production. In the enzyme producing fungus *Aspergillus niger* over 14000 genes were identified, using a trained gene-prediction algorythm and all were cheked manually. At this moment relevant fermentations are being sampled and studied using Affymetrix gene chips, 2D proteome gels, cDNA sequencing, etc. WWW5.DSM.COM

420. Improvement of foreign protein production by constitutive UPR induction in*Aspergillus niger var. awamori.* Mari Valkonen[1], Markku Saloheimo[1], Michael Ward[2] and Merja Penttilä[1] [1]VTT Biotechnology, P. O. Box 1500, FIN-02044 VTT, Finland [2]Genencor International, Inc., 625 Page Mill Rd., CA 94304-1013, USA

A signalling pathway from ER to nucleus called the unfolded protein response (UPR) controls the expression of genes for several endoplasmic reticulum-resident (ER) chaperones and foldases. When the unfolded protein response pathway is active, a signal is transduced from the ER lumen to the transcription machinery in the nucleus. It has been shown that the yeast transcription factor mediating the UPR induction of the chaperone and foldase genes is Hac1p. The UPR-induced form of the *A. niger* var. *awamori hacA* cDNA was expressed in *A. niger* var. *awamori* strain producing *Trametes versicolor* laccase. For the overexpression of *hacA*, the induced form of the *A. niger* var.*awamori hacA* cDNA was first created by deleting the 20 bp intron and truncating the 5' flanking region by about 150 bp, which omitted the upstream open reading frame. The laccase activity measurements made from the supernatants show that all the transformants produce more laccase than the parental strain. The production levels of the transformants were in the fifth day samples 3 to 7.6 fold higher than in the parental strain. On the seventh day of cultivation the transformants produced 2 to 5.4 fold more laccase than the parental strain. This indicates that the overexpression of HACA protein induces the production of heterologous proteins in *A. niger*.

421. Preliminary results of using *Aspergillus nidulans* microarrays to monitor gene expression in recombinant protein producing strains. Andrew Sims[1], Manda E. Gent[1], Geoffrey Robson[1], Nigel Dunn-Coleman[2], Rolf Prade[3], Hugh Russell[3], Stephen G. Oliver[1]. [1]School of Biological Sciences, Stopford Building, University of Manchester, Manchester, UK.,[2]Genencor International Inc, 925 Page Mill Road, Palo Alto, CA 94304, USA.,[3]Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA

The genus of filamentous fungi, *Aspergillus* has a high capacity for producing large amounts of secreted proteins, a property that has been exploited for commercial production of recombinant proteins. However the secretory pathway, which is key to the production of extracellular proteins is rather poorly characterised. The use of microarrays in the analysis of gene expression is becoming widespread for many organisms. Although a number of filamentous fungi have been fully or partially sequenced, microarray analysis is still in its infancy for these organisms. Aspergillus nidulans microarrays have been produced and validated in our laboratory. Work has now begun using these cDNA arrays with a range of different approaches to profile the effects of recombinant protein secretion on gene expression levels. Batch shake flask cultures have shown that chaperones such as binding protein (bipA) and protein disulphide isomerase (pdiA) are over expressed during production of the industrially and economically relevant recombinant protein, bovine chymosin. Chemostat fermentations have also been performed to compare gene expression in recombinant protein producing and wildtype strains during steady-state growth.

422. Cellular responses to secretion stress in *Trichoderma reesei*. Markku Saloheimo, Tiina Pakula, Mikko Arvas, Mari Valkonen and Merja Penttilä VTT Biotechnology, P.O Box 1500, 02044 VTT, Espoo, Finland

Trichoderma reesei is known for its extremely high capacity of protein secretion. High loads of protein, and especially foreign protein, in the secretory pathway form a challenge to the production organism and expose it to secretion stress. Unfolded protein response (UPR) denotes the induction mechanism of genes encoding ER-resident chaperones and foldases and numerous other genes involved in protein secretion. This induction is triggered when unfolded proteins accumulate into the ER. We have cloned the transcription factor involved in UPR induction, HACI, from *T. reesei*. Our results indicate that the *hac1* gene is activated by a dual mechanism operational at the mRNA level. This mechanism includes a splicing event of an unconventional intron of only 20 nt in length and a truncation of the mRNA at the 5' flanking region. This truncation removes an upstream open reading frame from the mRNA, and we have shown that these uORFs are involved in translational control of the HAC1 protein formation.

We have observed that concurrently with the induction of the UPR pathway, the genes encoding secreted proteins are rapidly down-regulated in *Trichoderma reesei*. This type of regulation can be caused by different secretion inhibitors and by foreign protein expression. The down-regulation is dependent on the promoter of the affected gene, suggesting that it is functional at the transcriptional level. The down-regulation of genes encoding secreted proteins during secretion stress has not been reported before from any other experimental system and thus it could be unique for filamentous fungi.

423. Genomic Organization, Transcript Analysis and Heterologous Expression of a Gene Family Encoding Cu-radical Oxidases (CRO) in the White Rot Basidiomycete *Phanerochaete chrysosporium*. Amber Vanden Wymelenberg [1], Philip Kersten [2], Robert Blanchette [3] and Daniel Cullen [1,2] [1] Department of Bacteriology, University of Wisconsin-Madison, USA, [2]USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin, and[3]Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota.

The white rot basidiomycete *Phanerochaete chrysosporium* is able to efficiently degrade all major components of wood: cellulose, lignin and hemicellulose. The oxidative enzymes thought to be involved in lignin degradation by this model system include lignin peroxidases (LiP), manganese peroxidases (MnP), and the peroxide-generating enzyme glyoxal oxidase (GLOX). Recently, a draft *P. chrysosporium* genome sequence has been made publicly available (http://www.jgi.doe.gov/programs/whiterot.htm). Blast searches of this database unexpectedly revealed at least five additional GLOX-like sequences. Transcripts were detected in various defined culture media and from *P. chrysosporium*-colonized wood. Full length cDNAs were cloned and sequenced. Comparisons of these sequences to GLOX and to related Cu-related oxidases showed close structural similarities around the active site. Interestingly, three highly conserved CRO sequences are located within a LiP gene cluster. Designated *cro3, cro4,* and *cro5,* all three feature a conserved N-terminal putative carbohydrate binding domain. Active enzyme corresponding to *cro3* has been produced in *Aspergillus nidulans* under the control of the *A. niger* glucoamylase promoter. Substrate specificity of the *cro3* protein is under investigation.

424. Role of the *bga1*-encoded extracellular beta-galactosidase of *Hypocrea jecorina* in lactose metabolism and cellulase induction. Bernhard Seiboth[1], Lukas Hartl[1], Noora Salovuori[2], Jari Vehmaanperä[2], Merja E. Penttilä[2] and Christian P. Kubicek[1]. [1] Institute of Chemical Engineering, TU Wien, Wien, Austria. [2] VTT Biotechnology, Espoo, Finland

Lactose is the only economic carbon source for protein production under the control of cellulase promoters by the ascomycete *H. jecorina* (anamorph: *Trichoderma reesei*). However, the mechanism by which lactose triggers cellulase formation is not understood. We have investigated the role of beta-galactosidase in lactose metabolism and cellulase induction in *H. jecorina*. A genomic copy of the *bga1* gene predicting a protein with a MW of 111 kDa (incl. a signal sequence) was cloned using degenerate primers. The Bga1 belongs to the Glycosyl hydrolases family 35. Transcriptional analysis of the *bga1* expression shows that it is highly expressed on L-arabinose, D-galactose and lower on lactose. Deletion of the gene showed that it is not essential for growth on lactose but that knock-out strains grew slower and produced about half the biomass of the wt strain, whereas amplification of the *bga1* gene under the *pki1* promoter resulted in faster growth with a reduced lag phase. Bga1 is not necessary for lactose-dependent induction of *cbh1* gene expression in *H. jecorina* but its overexpression impairs lactose-induction of *cbh1* expression. Analysis of the deletion strain further showed that *bga1* encodes the major extracellular beta-galactosidase which is also partially cell-wall bound. An additional cell wall-bound beta-galactosidase activity could be shown but no evidence for an intracellular beta-galactosidase was obtained.

425. Mutants of *Neurospora crassa* deficient in secreted protease activity. Edward B. Cambareri, Juan P. Montufar and W. Dorsey Stuart. Neugenesis Corporation, San Carlos, CA 94070-3389

Heterologous proteins produced by secretion in filamentous fungi are often quickly degraded by endogenous extracellular proteases. As part of our strain-improvement program we have induced, by UV as well as by integrative mutagenesis, a number of mutants that show decreased extracellular protease activity. Mutants were isolated by screening for colonies with decreased halo size on plates containing casein and gelatin. Chromatographic analysis demonstrated distinct loss of some species of media proteins that normally bind to a bacitracin protease affinity column. As reported by others, the genetic marker associated with the DNA mutagen does not always co-segregate with the mutant phenotype. Results of gene-disruption of a putative transcriptional regulator that may control secreted protease activities, related to the *prt-13* factor of *A. niger*, also will be presented.

426. Cytochrome P450 system genes in the white rot fungus *Phanerochaete chrysosporium:* cloning, differential regulation, and expression. Jagjit S. Yadav, P. Mishra, M. Safaie, V. Subramanian, and H. Doddapaneni Molecular Toxicology Division, Department of Environmental Health, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267-0056, USA

P. chrysosporium has been widely studied for its unique ability to biodegrade the plant polymer lignin and a broad range of recalcitrant organic pollutants, with a focus on its exo-oxidases. Lately, cytochrome P450 enzyme systems have been reported to be critical in several of these biotransformations. Characterization of the P450 systems will help understand overall metabolic pathways of biodegradation in this and other white rot fungi and their potential use in environmental or industrial applications. Our efforts have included cloning of the first three complete P450 monooxygenase genes, PC-1, PC-2, and PC-3 and their 2 splice variants (Fungal Genet. Biol.) and the P450 reductase gene, POR (Curr. Genet. 2000). Subsequently, recent whole genome sequencing has led to the identification of over 150 cytochrome P450 genes in this organism, the highest among lower eukaryotes. Real-time quantitative RT-PCR analysis showed that PC-1 is differentially regulated by nutrient levels; the transcript level was several fold higher in nitrogen-limited cultures than in N-rich or N- and C-rich cultures. POR expression did not seem to be as tightly regulated by the N & C conditions. Temporal analysis showed that the cloned genes are expressed in both primary and secondary metabolic phases of growth. PC-1 transcription was shown to be induced in presence of alkyl-substituted aromatics. The POR gene was heterologously expressed in active form in *E. coli* and the recombinant enzyme (82 kDa) was purified. Transcriptional analysis also showed its expressibility in *S. cerevisiae*. In an initial microarray analysis, about 30 of the selected 86 P450 monooxygenase genes were shown to be transcriptionally expressed.

427. Production of full-length antibodies and antibody fragments in *Aspergillus niger*. H. Wang, C Lin, D. Victoria, B. Fox, B. Fryksdale, D. Wong, H. Meerman, J. Pucci, M. Heng, X. Wang, R. Fong, and M Ward. Genencor International, Palo Alto, CA

Genencor International has previously developed methods for efficient secretion of foreign proteins in filamentous fungi, including *Aspergillus niger*. We have now demonstrated production of full-length humanized IgG1 and Fab' in *A. niger*. Both light chain and heavy chain were fused to the catalytic domain of the glucoamylase in separate plasmids and co-transformed into *A. niger* strains that were deleted for the glucoamylase gene. Antibody was subsequently cleaved off from the glucoamylase throught the engineered kex2 process sites. Yields of humanized IgG1 are competitive with those in mammalian cell systems and much higher than previously reported for other microbial hosts. The composition of the N-linked glycan added to the Fc region of human IgG1 by *A. niger* is of the high-mannose type and thus differs from the complex glycan structure of mammalian cells. We believe that *A. niger* will provide an attractive alternative for manufacturing some therapeutic antibodies.

428. Analysis of heterologous expression from *Aspergillus nidulans gpdA* and*alcA* promoters. David Lubertozzi and Jay D. Keasling, Dept. of Chemical Engineering, Univ. of Ca. Berkeley

Random integration of transforming DNA is common is *Aspergillus*, resulting in multiple copies throughout the genome. To study the effects of position, copy number, and promoter character on expression, *A. nidulans* strains were constructed with single- and multi-copy integrations of a plasmid bearing an expression cassette consisting of

an *Aspergillus alcA* (inducible), or *gpdA* (constitutive) promoter; the *E. coli lacZ* gene; and the *Aspergillus trpC* terminator. Homologous integration to the target loci was selected for by transformation with a truncated or mutated version of the wild-type genes, such that ectopic integration results in a null phenotype.

Integration of the *lacZ* gene was confirmed by PCR amplification of a fragment of*lacZ* and of the *argB* gene as control. Genomic locus and copy number were determined by Southern blotting of genomic restriction digests and probing with a^{32}P-labeled *lacZ* fragment. A rapid method to quantify transgene dosage utilizing real-time PCR was developed. A single-copy native gene (*trpC*) and *lacZ* were simultaneously amplified from genomic template from the transformants; by comparing the product curves to a series of standards, the amount of starting template DNA and hence gene copy number was determined. A tentative linear relation appeared for beta-galactosidase expression levels versus *lacZ* copy numbers, excepting a group of high expressing clones at ~10 copies.

429. Structural and expression analyses of retrotransposons from *Aspergillus oryzae*. Katsuya Gomi and Motohiro Sato. Agriculture, Tohoku University, Sendai, Japan.

We found an EST clone of *Aspergillus oryzae* highly homologous to the gene encoding reverse transcriptase (RT) of the LINE-like DNA element in a fungus, *Ascobolus immersus*. This EST clone seemed to be transcribed from the gene in an *A. oryzae* LINE-like element, and to be involved in its transposition through an RNA intermediate. Southern blot analysis showed that *A. oryzae* RIB40 has two copies of the DNA fragment hybridizing to the EST clone. Screening of the genomic library with the EST clone as a probe resulted in isolation of two positive phage clones, which are derived from different chromosome loci. Sequencing analysis of the DNA fragments inserted in the isolated phage clones revealed the existence of two different types of the LINE-like element, designated *Aoret1* and *Aoret2*. One phage clone contained whole putative retrotransposon, *Aoret1*, and the other had *Aoret2* about 6-kb downstream of Aoret1. Both putative retrotransposons contained two long ORFs encoded on the strand of the same direction. First ORF (ORF1) encoded a putative protein containing cysteine-rich motifs near the C-terminal region commonly found in gag-like proteins. The second ORF (ORF2) encoded a protein homologous to the RT. Northern blot analysis using a respective ORF2 of both retrotransposons as a probe revealed that major transcripts of approximately 5.5-kb in length were transcribed from the both retrotransposons. The transcripts were somewhat larger than the expected size for ORF2, but coincided with the full-length transcripts used as a LINEs RNA transposition intermediate. Thus if two proteins, gag-like protein and RT protein, would be produced, they might be translated from a single full-length transcript.

Population Biology Abstracts

430. Environmental and Clinical Populations of *Cryptococcus neoformans.* Anastasia P. Litvintseva[1], Rytas Vilgalys[2], and Thomas G. Mitchell[1].[1]Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710, [2] Department of Biology, Duke University, Durham, NC 27708

Cryptococcus neoformans is a basidiomycetous yeast and opportunistic pathogen of humans. Little is known about the genetic structure of environmental populations of this important pathogen. We collected over 600 environmental isolates of *C. neoformans* from North Carolina. To genotype these strains, we generated amplified fragment length polymorphism (AFLP) markers. We compared the data obtained for the environmental isolates with clinical isolates of *C. neoformans* from the same areas. We also compared them with environmental and clinical isolates from India and clinical isolates from Botswana. Among the North Carolina populations, AFLP analyses revealed that 81% of the isolates were serotype A and represented by a total of seven distinguishable AFLP genotypes, 8% were serotype D and represented by four distinct patterns, and 11% were serotype AD hybrids represented by five different genotypes. Analyses of the AFLP patterns by the UPGMA using arithmetic means revealed that the serotype A strains from North Carolina grouped into two well-separated clusters. Most of the clinical isolates of serotype A from North America, as well as environmental and clinical isolates from India were associated with either of these clusters. The Botswanian isolates were significantly different from both the North American and Indian populations of serotype A.

431. Sex and recombination in an imperfect world : evidence of both in Fusarium oxysporum. Keith Klein*, Veronique Edel-Hermann, Nadine Gautheron, and Christian Steinberg, , INRA UMR BBCE/IPM Dijon, France. *Dept. Of Biological Sciences, Minnesota State University, Mankato, Minnesota USA

A population sample of nonpathogenic *Fusarium oxysporum* from soil and plant roots was collected in 1991-92. This sample was characterized for intragenic spacer region (IGS) of rDNA by PCR-RFLP. The most numerous IGS type (106 isolates) was characterized for vegetative compatibility groups (VCG). The 106 strains analysed fall into 50 VCG types, of which 16 are multi-membered. These strains were characterized for mating type by PCR with primers specific for each of the two idiomorphs. 101 type 2 and 5 type 1 strains were found. 3 of the type 1 strains shared VCG with one or more type 2 strains. RFLP analysis of 45 of these strains was also performed. All possible recombinant types were found in the sample. These observations support the hypothesis of genetic recombination in this species. The presence of two mating types suggests that sexual recombination may be present, but parasexual recombination cannot be ruled out. The highly skewed sex ratio implies that sex is infrequent. 10 strains in the sample with the same VCG, mating-type and RFLP patttern were isolated from roots of wheat plants in a single year. This VCG was not found elsewhere, although other VCGs with wider distributions were found wheat. The RFLP pattern was shared,with minor variations, by all but three of the strains isolated from wheat. This suggests that selection at the gene level may be important in the association of non-pathogenic *F. oxysporum* with specific hosts.

432. Population studies of *Pythium* species causing greenhouse diseases. Carla D. Garzon, David M. Geiser and Gary W. Moorman Department of Plant Pathology, The Pennsylvania State University

The population genetics of *Pythium* species is a little explored area of research. Most studies have focused on *P. ultimum*, but there is no reason to consider this species as a representative model for the genus. Other important plant pathogenic *Pythium* species, including *P. irregulare* and *P. aphanidermatum*, have attracted little attention from geneticists despite the fact that they can be aggressive and devastating pathogens on greenhouse crops. We performed preliminary studies to evaluate the genetic diversity of *P. aphanidermatum* and *P. irregulare* using AFLP genetic markers produced by a single selective primer combination. The results of these studies indicated that these two species show patterns of variation very different from that observed in *P. ultimum* and from each other. Nineteen *P. irregulare* isolates from the US showed species structure, being divided into two groups of overlapping geographical distribution. On the other hand, *P. aphanidermatum* showed very little genetic variation worldwide, based on an analysis of 23 isolates from Asia, Africa, North and South America. To examine the population genetics of *P. irregulare* and *P. aphanidermatum* and to identify AFLP genetic markers associated to fungicide resistance in isolates from the United States, ninety *P. irregulare*and 70 *P. aphanidermatum* isolates were analyzed using seven additional selective AFLP primer combinations. We report our current findings and conclusions.

433. Evolutionary aspects of gibberellin biosynthesis in the *Gibberella fujikuroi* species complex. Stefan Malonek and Bettina Tudzynski. Westfälische Wilhelms-Universität Münster, Institut für Botanik, Schloßgarten 3, D-48149 Münster, Germany

G. fujikuroi is a species complex of at least 36 distinct *Fusarium* species (section Liseola) with monophyletic origin. On the basis of sexual compatibility the species complex was divided into 8 mating populations (MP-A to MP-H). In contrast to the other MPs, only members of MP-C (*F. fujikuroi*), pathogenic on rice, produce high amounts of gibberellins (GAs), resulting in abnormous growth of rice seedlings. The seven genes involved in the biosynthetic pathway were found to be organised in a gene cluster in MP-C. Except for MP-A (*F.moniliforme*), which have only two genes left, strains of the other MPs consist of all seven genes of the cluster which are highly homologous (90-98%) to the corresponding sequences of MP-C. However, only the two genes of MP-A were shown to be highly expressed under GA producing conditions so far. In order to find the reason(s) for the lost ability to produce GAs, we started a detailed molecular characterisation of the GA biosynthetic genes from two MPs, MP-D (*F.proliferatum*) and MP-A, including sequencing, expression studies, cluster organisation and functional analysis. For this task, the genes were transformed into a mutant strain of MP-C which has lost the entire gene cluster due to a big deletion, but still has all the regulatory genes for GA biosynthesis. First results revealed full functionality of the so far analyzed genes of MP-D in the MP-C background. However, feeding of radiolabelled intermediates gave evidence for a genetic block at the step of *ent*-kaurene oxidation catalyzed by the gene *P450-4*. This gene of MP-D is now under detailed investigation to find the reason for non-functioning. Thus we want to investigate the evolutionary

developement of this big species complex of *G. fujikuroi* using the dispensable gene cluster for the GA biosynthetic pathway as a molecular marker.

434. Exploring the fitness of filamentous fungi using a lichen, *Xanthoparmelia cumberlandia*, and *Neurospora crassa* as model systems. A. Pringle and J.W. Taylor. UC Berkeley, Plant & Microbial Biology, Berkeley, CA

There is no standard measure of fungal fitness. Mycologists have choosen to understand fitness as either a combination of survival and reproduction, or as a comparative variable which contrasts the reproductive success of two individuals. We argue that fitness should be understood as the combination of survival and reproduction, equivalent to: $R = *l_x m_x$ where R is fitness, l_x is the probability of survival to age x, and m_x is the number of progeny produced by an individual at age x. Measuring R is laborious; it may be useful to choose a single aspect of fitness as a surrogate of R. Choosing which aspect of fitness to measure can be facilitated by an understanding of how fitness measures are correlated, unfortunately, few data record the correlations between different aspects of fitness. In two separate studies we demonstrate significant correlations between different aspects of fitness. First, *Xanthoparmelia cumberlandia* is used to demonstrate a significant correlation between thallus size and reproductive effort, showing that size is an easily measured surrogate of fitness. Published data of other species show a similar correlation, indicating that it may be a general feature of sexual lichens. Second, *Neurospora crassa* is used to explore correlations between spore germination, mycelial growth, biomass, and sexual and asexual sporulation.

435. The human pathogen *Aspergillus fumigatus* is a single globally distributed fungal species. A. Pringle, D. M. Baker, J. L. Platt, J. P. Wares, J.P. Latge and J.W. Taylor. UC Berkeley, Plant & Microbial Biology, Berkeley, CA

Aspergillosis is a typically fatal fungal infection of immunocompromised humans caused by species in the genus *Aspergillus*, including *A. fumigatus*. The fungus is assumed to be asexual as neither mating, sexual structures, nor meiospores, have been observed. Investigations of the species status and reproductive habit of other fungi have shown that morphological species may harbor cryptic genetic species, and that apparently asexual fungi can be sexual. Understanding whether or not *A. fumigatus* comprises multiple species, and is cryptically sexual, is a matter of biological interest; the data will also provide critical information to the medical community, which must manage the evolution of resistance to widely prescribed drugs and design effective anti-fungal therapies. Sixty-one cultures of *A. fumigatus* were collected from Africa, Asia, Europe, and North and South America. Using a phylogenetic species concept, we analyzed sequence data of five microsatellite loci and conclude that *A. fumigatus* is a single globally distributed fungal species. We are currently using these same data to investigate the sexuality of the fungus.

436. Creation of Congenic Serotype A Isolates of *Cryptococcus neoformans.* S.M. Keller[1], M.A. Viviani[2] and B.L. Wickes[1]. [1] Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA [2]Laboratorio di Micologia Medica, Istituto di Igiene e Medicina Preventiva, Universita degli Studi, IRCCS Ospedale Maggiore, Milano, Italy

Cryptococcus neoformans, an opportunistic human fungal pathogen, is comprised of two varieties (*neoformans* and *gattii*) and 4 serotypes (A, B, C, D). Recently, the first fertile serotype A *MATa* isolate (IUM 96-2828) was described in detail, making classical genetic analysis using serotype A strains, the most frequent clinical isolate, possible. Congenic strains are beneficial for mating studies and to clean up the backgrounds of gene disruptant strains for *in vivo* testing. At present, only congenic serotype D strains are available for use in classical genetic analysis, which was the major reason why this serotype was chosen for the genome sequencing project. Using H99 (standard serotype A *MATalpha* laboratory isolate) and IUM 96-2828 (serotype A *MATa* environmental isolate) as parents, pairs of serotype A congenic strains were prepared in each background by successive backcrossing through 10 generations. Each F_{10} congenic strain was compared to its respective parent strain using AFLP analysis. Preliminary data suggests that these strains differ only at the mating type locus. Genetic markers were created and then moved into these congenic pairs of strains to facilitate efficient mating and to provide host strains for transformation. The serotype A congenic strains created in this study will prove a valuable tool for addressing questions about mating type and the effects of genetic background on characteristics like virulence.

437. Nuclear reassortment between vegetative mycelia of the basidiomycete *Heterobasidion annosum* in nature. Hanna Johannesson[1] and Jan Stenlid[2].[1] Department of Plant and Microbial Biology, Berkeley, USA.[2] Department of Forest Mycology and Pathology, Uppsala, Sweden.

The recognition of self from non-self in basidiomycetes is mediated by the somatic incompatibility (SI) system, a multigenic system that prevents free exchange of nuclei and cytoplasm between heterokaryotic individuals of a population. SI in the basidiomycete*Heterobasidion annosum* is controlled at a series of three to four multiallelic discrete loci. Previous laboratory studies have indicated that SI reactions are not an absolute block to nuclear exchange between unrelated heterokaryotic strains of *H. annosum*, and in this study we present evidence for nuclear reassortment between vegetative mycelia in natural populations of the species. By using six highly variable microsatellite markers we genotyped single nuclei obtained from 18 somatically incompatible heterokaryotic strains of *H. annosum* originating from a single stump of *Picea abies*. We found that 28 nuclei compose the 18 heterokaryotic genotypes found in the stump; 19 of the nuclei were found in more than one heterokaryotic mycelia. Furthermore, in one of the heterokaryotic mycelia we verified the coexistence of four different nuclei. Heterokaryotic single-conidial mycelia, each containing two nuclei from that mycelium in different combinations, were somatically incompatible when paired on Petri-dishes. These results show that the SI system is not preventing nuclear migration in heterokaryotic mycelia of *H. annosum*.

438. Genetic diversity of *Fusarium graminearum* from maize in Korea. Jae-Jin Jeon[1], Hun Kim[1], Hye-Sun Kim[1], Kurt A. Zeller[3], Theresa Lee[1], Sung-Hwan Yun[2], Robert L. Bowden[3], John F. Leslie[3], and Yin-Won Lee[1].[1]School of Agricultural biotechnology, Seoul National University, Suwon 441-744, Korea, [2]Division of Life Sciences, Soonchunhyang University, Asan 336-745, Korea, [3]Department of Plant Pathology, 4017D Throckmorton Plant Sciences Center, Kansas State University, Manhattan KS 66506-5502.

A total of 584 isolates of *Fusarium graminearum* (Shwein.) Petch (teleomorph:*Gibberella zeae*) were obtained from maize in Gangwon province of Korea during 1999-2000. Of these isolates, 500 were self-fertile and 84 were female-sterile. A phylogenetic tree of the isolates was constructed by using amplified fragment length polymorphism (AFLP). AFLP showed polymorphic bands and these bands, haplotypic loci, were used to analyze population genetic diversity. Population structure of the isolates consists of four lineages (7, 6, 3 and 2). Lineage 7 was the major group (74%) and followed by lineage 3 (13%), lineage 6 (12%) and lineage 2 (1%). Estimate of Nei¡¯s *Gst* and *Nm* values showed a significant difference in allelic frequencies among lineages. Maximum parsimony trees based on selected sequencing data from *Tri101*, *Tri7* and *MAT-1-1-1* genes was found to be concordant with AFLP data. Each lineage showed a significant difference in fertility. Fertility of lineage 7 isolates was 100% followed by lineage 6 and lineage 3 isolates that showed 69% and 25%, respectively. When the representative isolates of each lineage were inoculated to barley, they were virulent regardless of lineages. Trichothecene production of *F. graminearum* isolates was variable; lineage 7 and lineage 3 isolates produced deoxynivalenol (DON), whereas lineage 6 and lineage 2 isolates produced nivalenol (NIV). The results of this study provide the difference of genetic variation among lineages in *F. graminearum* population from maize in Korea.

439. Molecular Phylogenetic Analysis Indicates *Cephalosporium maydis* a Distinct Taxon in the *Gaeumannomyces-Phialophora* Complex. A.A. Saleh[1] and J.F. Leslie[2]. [1]Agricultural Genetic Engineering Research Institute, ARC, Giza, Egypt. [2]Dept. of Plant Pathology, Kansas St. University, Manhattan, KS 66506.

A recent hypothesis is that the maize pathogen *Cephalosporium maydis* is closely related to, if not a part of, the *Gaeumannomyces-Phialophora* (GP) complex. We evaluated strains of *C. maydis*, other *Cephalosporium* spp., and the GP complex using several DNA-based techniques. Based on AFLP data, *C. maydis* is a unique species that is distinct from other plant pathogenic species of *Cephalosporium* and*Gaeumannomyces*. We obtained DNA sequences of four nuclear genes from four strains representing *C. maydis* and 21 additional, potentially related, species. These data are consistent with the hypothesis that *C. maydis* is a distinct taxon that is closely related to the GP complex. Although no sexual stage is known for *C. maydis*, the *MAT-2*allele is > 77% similar to *MAT-2* sequences from other *Gaeumannomyces* strains. The clade formed by strains from the GP species complex was monophyletic in our analysis, but the clade formed by strains from the *Acremonium-Cephalosporium* (AC) complex was not. We also found that *C. gramineum*, the causal agent of Cephalosporium stripe disease of wheat, is distantly related to both the AC and the GP species complexes. Thus the taxonomic treatment of both *C. maydis* and *C. gramineum* needs revisions, with *C. maydis* assigned to the GP complex and *C. gramineum* assigned to another genus.

440. Biological Species in the *Gibberella fujikuroi* species complex (*Fusarium* section *Liseola*) recovered from Maize and Sorghum in Egypt. A.A. Saleh and J.F. Leslie. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

Fusarium species in section *Liseola*, with teleomorphs in the *Gibberella fujikuroi* species complex, cause stalk, ear and kernel rots of maize and sorghum and produce mycotoxins such as fumonisins and moniliformin. We have examined 353 isolates within section *Liseola*, isolated from both maize and sorghum. Species among these isolates were identified with AFLP markers and sexual fertility testing. We recovered representatives of *G. fujikuroi* mating populations (MPs), MP-A (*F. verticillioides*, teleomorph *G. moniliformis*), MP-D (*F. proliferatum*, teleomorph *G. intermedia*), MP-F (*F. thapsinum*, teleomorph *G. thapsina*), and MP-G (*F. nygamai*, teleomorph *G. nygamai*), along with members of an undescribed biological species closely related to *F. andiyazi*. MP-A was the most frequently recovered MP from maize (73% of recovered isolates), and MP-D was the most frequently recovered MP from sorghum (51% of recovered strains from sorghum). Female fertile strains were most common within MP-A (68%). The inbreeding effective population sizes (Ne), based on mating type frequencies, for MP-A, MP-D, and MP-F were 85%, 100%, and 88%, respectively. Based on the observed frequency of female fertility, estimated Ne of the MP-A, MP-D, and MP-F isolates were 98%, 70%, and 31%, respectively. Our results suggest that sexual reproduction occurs more frequently within MP-A than within MP-D or MP-F. The relatively low female fertility within MP-D and MP-F may limit genetic exchange among individuals within these species relative to that possible in MP-A.

441. Might the B-alpha and B-beta mating-type gene products of *Schizophyllum commune* really cross-talk? Thomas J. Fowler, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT USA 05405

Two of the four loci that determine mating type in the heterothallic mushroom-bearing fungus *Schizophyllum commune* are the linked and redundantly functioning multigenic loci called B-alpha and B-beta. Within the species there are multiple versions of both B-alpha and B-beta. Until now, only portions of a few of the versions of these loci had been cloned, sequenced, and characterized in biological assays. DNA containing the linked B-alpha3-B-beta2 versions has been sequenced. Eleven genes encoding lipopeptide pheromones and two genes encoding seven-transmembrane domain pheromone receptors are located in this B-mating-type complex. All of these genes have been confirmed to confer mating activity. It had always been thought that pheromone and receptor components encoded by B-alpha do not activate or respond to the pheromone and receptor components encoded by B-beta, and vice versa. However, some pheromones encoded by B-beta2 do activate receptors associated with the B-alpha versions B-alpha8 and B-alpha9 in addition to activating B-beta receptors. The B-alpha8 receptor gene was subsequently isolated and shown to predict a receptor very similar to the B-beta1 receptor. These findings indicate that some cross-talk between the signaling components encoded by the two loci probably does occur, but may also indicate that the B-complexes containing B-alpha8 and B-alpha9 do not consist of a simple alpha-beta pair. Previously published studies of recombination between B-alpha and B-beta support this latter idea, but more molecular information will be required for confirmation. And where do these cross-talking versions fit into the evolutionary history of the *S. commune* B-loci?

442. Molecular diversity between maize and banana populations of *F. verticillioides*. Mulè G. , Mirete, S., M.T. González-Jaén, A. Moretti, C. Vázquez, B. Patiño, A. Logrieco. Is. Scienze Produzioni Alimentari, Bari, Italy, and Univ. Complutense Madrid, Madrid, Spain

Fusarium verticillioides is one of the most prevalent Fusarium species on maize, causing stalk and ear rot worldwide. The organism, associated with various animal diseases, is able to produce potent mycotoxins, the fumonisins. A population morphologically identified as *F. verticillioides* has been also reported from banana fruits. This latter population showed different toxigenic profile, being able to produce in vitro moniliformin and not fumonisins, and although produced fertile perithecia of mating population A, time for obtaining perithecia and size of them differed significantly from usual fertile crosses among strains from maize. Two different molecular approaches, amplified fragment length polymorphism (AFLP) and the TEF-a (translational elongation factor alfa) were used to strengthen these differences. All molecular methods showed two different clusters, supporting the fact that they represent two distinct populations.

443. Phylogeny and Biogeography of *Morchella*. Kerry O'Donnell[1], Nancy S. Weber[2], Steve Rehner[3] and Gary Mills[4]. [1]USDA/ARS/NCAUR, Peoria, IL,[2]Oregon State University, Corvallis, OR,[3]USDA/ARS/BARC, Beltsville, MD and [4]Michigan State University, East Lansing, MI.

Species of the filamentous ascomycete genus *Morchella*, better known as the true morels or morel 'mushrooms', are among the most highly-prized macrofungi collected by mycophiles during Spring in the northern hemisphere. Field guides to morels typically recognize 6 or fewer morphospecies and these are generally assumed to be cosmopolitan in their distribution. To test these hypotheses, multigene genealogies were constructed from a global collection of approximately 600 individuals to investigate the phylogeny and biogeography of*Morchella*, using a genealogical concordance version of phylogenetic species recognition. Early in the study, allelic variation within the nuclear rDNA internal transcribed spacer region was used as a genetic screen for the detection of putative phylogenetically distinct species. Parsimony analysis of the combined multilocus DNA sequence data provided a nearly fully resolved phylogeny in which a monophyletic *Morchella* comprised two sister clades: the 'yellow-tan-grey' morels (*Esculenta* clade) and the 'black' morels (*Elata* clade) with 13 and 15 species, respectively. Biogeographical interpretation of the phylogenetic data suggests that the ancestral area for *Morchella* is North America, the most phylogenetically diverse area studied with 13 endemics [4 *Esculenta* clade, 9*Elata* clade]. The most surprising result of this study is that at least 24 of the 28 species exhibit continental endemism. Results of this study have practical implication for morel breeding and conservation biology and provide a robust phylogenetic framework for studying the evolution of mating systems.

444. *Hop*,an active *Mutator*-like element isolated in *Fusarium oxysporum* transposes in the heterologous species *Fusarium culmorum*. Fabienne Chalvet, Christelle Vasnier and Marie-Josée Daboussi Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France

A new type of active DNA transposon has been identified in the genome of *Fusarium oxysporum*through its transposition into the *niaD* target gene (Chalvet et al.). Its structural features (length, ITRs size, 9 bp duplication) as well as the presence of conserved domains in the transposase are those characterizing *Mutator*-like elements (MULEs). Therefore, *Hop* is the first active member of this family found outside the plants. The high rate of excision observed in spite of its location in an exon indicates that *Hop* is very active and thus represents a promising efficient tagging system for fungal gene isolation. The*Hop* copy inserted in the *niaD* gene has been introduced in different genetic contexts free of endogenous elements in order to check for its autonomy. Evidence for excision was found in *Fusarium culmorum*. The properties of transposition of such element (reinsertion frequency, target site duplication, propensity to insert into genes) are under investigation. The fact that *Hop*-related elements were found in different distant related species suggest that *Hop* would be able to function in a wide range of ascomycetes.

445. Tracking DNA polymorphisms in field populations of *Aphanomyces cochlioides*. John J. Weiland, USDA-Agricultural Research Service, Red River Valley Agricultural Research Center, Fargo, N.D 58105

Root and seedling diseases caused by *Aphanomyces cochlioides* are serious impediments to sugarbeet production in wet growing regions, yet information on the genetics and the inheritance of virulence in this organism is lacking. No race structure for *A. cochlioides* has been reported and preliminary studies have revealed limited genetic diversity in this oomycete using DNA-based technologies. In the present study, application of random amplified polymorphic DNA (RAPD) analysis to single zoospore isolates obtained from sugarbeet fields in the U.S. identified 2 polymorphisms that assorted randomly within local populations; some field populations harbored only one polymorphic type. The data indicate that these polymorphisms are found in *A. cochlioides* isolates ranging from the northern Red River Valley of the U.S. to the historic regions of sugarbeet production in Texas. Implications of this result in the development of novel virulence and fungicide resistance in *A. cochlioides* are discussed.

446. Population Genetic Differentiation and Lineage Composition Among *Gibberella zeae*in North and South America. K.A. Zeller[1], J.I. Vargas[1], G. Valdovinos-Ponce[1], J.F. Leslie[1], & R.L. Bowden[2]. [1]Dept. of Plant Pathology, Kansas State Univ., Manhattan, KS;[2]USDA-ARS Plant Sci & Entomology Res. Unit, Manhattan, KS.

G. zeae (*Fusarium graminearum*) causes Fusarium Head Blight (FHB) of wheat and barley, and has been responsible for severe economic losses worldwide. Sequence analyses of *G. zeae* have been interpreted to mean that

populations of *G. zeae* are composed of eight phylogenetic lineages, with a phylogeographic structure among these lineages. We compared AFLP polymorphisms in populations of *G. zeae* from the United States, Mexico, Brazil, and Uruguay. Populations of *G. zeae* causing FHB in the United States include only a single phylogenetic lineage (lineage 7). Subpopulations across the United States have high genotypic diversity, do not deviate from expectations of random mating, and are interconnected by extensive gene-flow. South American populations of *G. zeae* from both wheat and from sorghum include a minority component of isolates that cluster with other phylogenetic lineages (lineages 1, 2, and 6), but are dominated by genotypically diverse populations of isolates from lineage 7. Populations of *G. zeae* causing FHB on wheat from two locations in Mexico are dominated by isolates from lineage 3. Intercontinental gene flow probably occurs from North to South America, but the amount of gene flow between the continents is much less than that occuring within each continent.

447. Interfertility and Marker Segregation in Hybrid Crosses of *Gibberella fujikuroi* and *Gibberella intermedia.* K.A. Zeller[1], M.A Wohler[1], L.V. Gunn[2], S. Bullock[2], B.A. Summerell[2] and J.F. Leslie[1]. [1] Department of Plant Pathology, Kansas State University, Manhattan, KS;[2] Royal Botanic Gardens, Sydney, NSW, Australia.

Gibberella fujikuroi and *Gibberella intermedia* [mating populations (MP) C and D of the *Gibberella fujikuroi* species complex] generally can be distinguished by differences in the spectrum of mycotoxins produced, the lack of sexual cross fertility, and diagnostic differences in DNA sequences. Some isolates from these two biological species, however, can interbreed and complete meiosis to produce viable progeny. Analysis of marker segregation amongst such hybrid progeny can be used to estimate the degree of genomic rearrangement and genetic incompatibility that has accumulated since these sibling species diverged. We isolated recombinant progeny from crosses of MP-C × MP-C, MP-D × MP-D, and MP-C × MP-D. These progeny segregate for the *MAT* locus, a homolog of the*Fum1* locus, and numerous loci defined by amplified fragment length polymorphisms (AFLPs). In intra-specific crosses, most of the AFLP loci segregate in the expected 1:1 ratios. In MP-C × MP-D crosses more of the AFLP loci appear to deviate from the expected 1:1 segregation ratios. We are evaluating the fertility of the hybrid progeny in backcrosses with their parents to identify genetic loci that are critical to the evolutionary differentiation of these two sibling species.

448. Genetic consequences of habitat fragmentation in rare wood decay fungi. Jan Stenlid, Mårten Gustafsson, and Nils Högberg Department of Forest Mycology and Pathology, SwedishUniversity of Agricultural Sciences, Box 7026, S-75007 Uppsala, Sweden.

Fragmentation of habitat is a major threat to species diversity in forest ecosystems. In boreal forests, many wood-inhabiting organisms, including fungi, are threatened due to scarcity of localities containing sufficient amount of substrates. We here present studies in two species with a fragmented habitat in parts of their natural distribution range. Based on variation in variable regions of coding genes and in RAPD profiles, studies in the rare polypore *Fomitopsis rosea* suggested a reduced heterozygosity in small populations in Southern Sweden and Russia, but heterozygosity was close to expectations in larger populations in Northern Sweden, Norway and Finland. Small populations may experience reduced fitness. In *F. rosea* and *Phlebia centrifuga*, spore germination was reduced in small populations outside the core range of distribution in Sweden. Dispersal in Basidiomycete fungi can be followed using a species specific spore trapping approach with homokaryotic mycelia. The dispersal range was correlated with the strength of spore source, and in a recent study, the spore dispersal outside the regional range in Sweden of fruiting bodies in *F. rosea* and *P. centrifuga* was undetectable. Future work will involve studies on the effect of inbreeding on several fitness parameters e.g. wood decay ability and interspecific competition.

449. Microsatellite evolution in *Neurospora*. Jeremy Dettman and John Taylor Plant and Microbial Biology, UC Berkeley

Microsatellite loci have become one of the most popular classes of markers for population genetic analyses. Despite this fact, the evolutionary dynamics and mutational processes of microsatellites are still not fully understood. To address this issue, we sequenced 4 unlinked microsatellites and their flanking regions from 147 strains of *Neurospora*. Representatives from eight phylogenetic species were included, with most emphasis on *N. crassa* and *N. intermedia*. To elucidate the genealogical relationships among alleles, repeat number was mapped onto trees constructed from sequence data. This allowed us to place the microsatellite mutations in the evolutionary context of the less rapidly evolving flanking regions. Within populations and species, distributions of allele repeat number were

generally consistent with the stepwise mutational model proposed for microsatellites. Indels in the flanking regions were observed, but they caused only small amounts of intraspecific allele length homoplasy, confirming the usefulness of microsatellites for population level analyses. While flanking sequences were quite divergent among species, allele repeat number distributions commonly overlapped, i.e., alleles that were identical in state, but not identical by descent, were shared among species. As well, several mutations in the tandem repeats themselves were observed within certain lineages. These high levels of interspecific homoplasy indicated that several more microsatellite loci must be characterized before we can test their efficacy in phylogenetic reconstruction among *Neurospora* species.

450. Sympatric biological and phylogenetic species among pseudohomothallic isolates identified as *Neurospora tetrasperma*. G. S. Saenz[1], D. J. Jacobson[2], W. H. Dvorachek[1], and D. O. Natvig[1].[1]University of New Mexico, Albuquerque, [2]Stanford University

Isolates assigned to *Neurospora tetrasperma* are distinct within the genus in possessing four-spored asci. Each large ascospore gives rise to a heterokaryotic (*mat A +mat a*), self-fertile thallus, which is pseudohomothallic. Our previous work showed that: 1) pseudohomothallism is monophyletic in *Neurospora*, and 2) outbreeding is common within populations of *N. tetrasperma*, despite predominant selfing and frequent sexual dysfunction during outbreeding. Phylogenetic and reproductive relationships were assessed among 14 strains of *N. tetrasperma* from one population in a single 5 ha field. Upstream regions of four genes, *het-c, frq, sod-2, and pdx-2* were sequenced. Reproductive isolation was assessed by outcrossing. Tree-building analyses revealed three separate, well-supported sympatric phylogenetic species within this local population. However, only one of the three lineages was reproductively isolated in laboratory crosses. The results suggest that phylogenetic differentiation can precede the development of genetic barriers to sexual reproduction, as also seen in *N. crassa* and *N. intermedia* (Dettman et al. FGN 49S:19). This implies a common speciation trend within *Neurospora*, but is particularly enigmatic for *N. tetrasperma* given the sympatric nature of the closely related putative species.

451. Phylogenetic, biogeographic and population genetic approaches to the analysis of cryptic speciation in the entomopathogen.

Stephen A. Rehner and Ellen P. Buckley. USDA/ARS, Insect Biocontrol Laboratory, Beltsville, MD.

Beauveria is a genus of globally distributed entomopathogenic hyphomycetes of practical interest as biological control agents of pest insects. Species recognition in *Beauveria* is difficult due to a lack of informative morphological structures. A gene-genealogical concordance approach to species recognition was used to investigate phylogenetic diversity of *Beauveria*, particularly *B. bassiana*, a cosmopolitan species widely used in biological control of agricultural pest insects. Phylogenetic analyses of 7 loci revealed five major clades, each of which was possessed fairly uniform spore characteristics, however the clades do not correspond well to prevailing species concepts for this genus. Most notably, the morphological species *B. bassiana* is biphyletic with morphologically similar strains divided among two unrelated clades. All clades consisted of multiple endemic lineages indicative of an underlying pattern of cryptic diversification. Biogeographic structuring in the *B. bassiana* complex is consistent with a mode of divergence in allopatry. Several *Cordyceps* species are derived from within Beauveria phylogeny, thus *Beauveria* species are either inherently sexual or, if strictly asexual, are likely to be recently derived from *Cordyceps*. Polymorphic microsatellite markers have been developed for the type *B. bassiana* clade for inferring population genetic structure and investigating speciation and species boundaries.

452. Identification of a mating-type gene in the homothallic fungus *Aspergillus nidulans*. Paul S Dyer, Mathieu Paoletti, Marcos J Alcocer and David B Archer. School of Life and Environmental Sciences, University of Nottingham, Nottingham NG7 2RD, UK.

Mating-type (*MAT*) genes have been identified from the pyrenomycete, loculoascomycete and discomycete classes of ascomycete fungi. By using hot-start PCR with degenerate primers, together with thermal asymmetric interlaced (TAIL)-PCR, it has been possible to identify a characteristic *MAT-2* gene from the plectomycete fungus *Aspergillus nidulans*. It includes a conserved high mobility group (HMG)-domain. RACE-PCR analysis has been used to investigate transcription of the gene, confirming the presence of an intron in a conserved position within *MAT-2*

genes. Further analysis of the flanking regions of the *MAT-2* gene has revealed three further genes, including a putative gene with homology to an element of the anaphase-promoting complex (APC) of *Schizosaccharomyces pombe*. The presence of an *APC* homologue provides evidence of microsyteny around the *MAT* locus. However, no *MAT-1*alpha-domain gene could be detected. This suggests either that a *MAT-1* homologue may be present elsewhere in the genome, not directly adjacent to the *MAT-2* locus, or that*A. nidulans* may contain only a *MAT-2* mating-type gene. This would be a unique situation among homothallic euascomycetes so far analysed, which contain either a*MAT-1* gene or both *MAT-1 /MAT-2* genes.

453. Evaluating the likelihood of genetic recombination between introduced and indigenous strains of the entomopathogenic fungus *Beauveria bassiana* in agricultural fields. Louela Castrillo[1], John Vandenberg[2], and Michael Griggs[2]. [1]Department of Entomology, Cornell University, Ithaca, NY 14853 and [2] USDA-ARS, US Plant, Soil and Nutrition Lab., Ithaca, NY 14853.

The fungal pathogen *Beauveria bassiana* is widely used as a mycoinsecticide for control of several insect pests, providing a biological alternative to synthetic chemical insecticides. A key advantage for microbial control agents is their potential to replicate and persist in the environment, offering continued suppression of insect pest populations. However, exploiting this advantage is commensurate with the need to determine the impact of mass releases of this fungus on non-target organisms and to assure safety and long-term efficacy. To date, no information is available on the potential for genetic recombination between introduced and indigenous strains of *B. bassiana* in agricultural fields and whether this can result in recombinants with altered virulence and host range. In this study we evaluated the likelihood of genetic recombination by determining 1) vegetative compatibility groups (VCG) among *B. bassiana* strains indigenous to the US and strains that have been mass released for insect control and 2) the frequency of recombination between co-infecting strains of *B. bassiana*in an insect host, where recombination is likely to occur in nature. Our data revealed a group of genetically similar strains isolated from Colorado potato beetles belonging to the same VCG. These strains originated from the northeastern part of the US and from Quebec and Ontario, Canada. Co-inoculations of beetle larvae with complementary *nit* mutants resulted in heterokaryon formation only between strains of the same VCG, suggesting that the self/non-self recognition system of the parasexual process is an effective barrier preventing genetic exchange between dissimilar strains in the field. Further studies are being conducted to determine stability and virulence of recombinants.

454. Population subdivision in *Fusarium graminearum* lineage 7 in the U.S. is correlated with toxin chemotype. Liane Rosewich Gale[1], Todd Ward[2], Virgilio Balmas[3], and H. Corby Kistler[1].[1]USDA-ARS, University of Minnesota, MN 55108, [2]USDA-ARS Microbial Genomics Research Unit, Peoria, IL 61604, [3]Università degli Studi di Sassari, I-07100 Sassari.

Isolates of *Fusarium graminearum*, causing head blight of wheat, from nine U.S. states, representing 86 fields in 53 counties, were characterized using ten single-copy RFLP probes, a telomeric probe and RFLP probes diagnostic for species and lineage. In addition, isolates were assigned to one of three profiles of trichothecene metabolites (chemotypes) using a PCR-based approach. Most of the 712 isolates examined were confirmed as lineage 7, though four isolates from ND had slightly different RFLP patterns and were excluded from further analysis. The telomeric probe was used for clone determination, leaving 587 isolates for subsequent data analyses. Most lineage 7 isolates (94.6%) from the U.S. were of 15acetyl deoxynivalenol (15ADON) chemotype. The 3acetyl deoxynivalenol (3ADON) chemotype was found at 5% and was only identified in samples from ND and MN. The nivalenol chemotype was infrequent at 0.4%. Gene flow analysis demonstrates that the 15ADON population in the U.S. is genetically isolated from the 3ADON population (Nm = 0.5). In comparison, a representative collection consisting of 19 isolates of lineage 7 from Italy was genetically similar to the 3ADON population from the U.S. (Nm > 2). Regarding diversity, no major differences could be discerned between the 3ADON and 15ADON populations from the U.S., even though the 3ADON population was nearly fixed at some RFLP.

455. Multi-gene phylogenies reveal taxonomic confusion in the genus *Magnaporthe*. Stephen B. Goodwin[1], Morris Levy[2], Jessica R. Cavaletto[1] and Yang Tian[3]. [1] USDA-ARS,[2] Department of Biological Sciences, [3] Department of Botany and Plant Pathology, 915 West State Street, Purdue University, West Lafayette, IN 47907-2054, USA

The genus *Magnaporthe* contains a number of species infecting monocot hosts. One of these, which causes blast of rice, formerly was called *M. grisea*. However, rice-infecting isolates of *M. grisea* were separated recently into the new species, *M. oryzae*. This analysis cleared up some taxonomic confusion, but did not address the relationships of the *M. grisea* complex and other species of *Magnaporthe* to related genera, such as *Gaeumannomyces*. To determine in more detail the phylogenetic relationships of *M. grisea*, the internal transcribed spacer region of isolates from diverse hosts was sequenced. Sequences from related species, primarily *Magnaporthe* and *Gaeumannomyces*, were downloaded from GenBank, along with sequences representing the Diaporthales as an outgroup. Neighbor-joining analysis revealed that the genus*Magnaporthe* was polyphyletic; *M. poae*, *M. rhizophila* and the type species for the genus, *M. salvinii*, all clustered within *Gaeumannomyces* rather than with *M. grisea* and *M. oryzae*. The species *M. grisea* comprised a monophyletic group of several genetically distinct taxa that are mostly host delimited. In addition to *M. oryzae*, clusters with high bootstrap support that may represent separate species include: most isolates from *Cenchrus*/*Pennisetum*; isolates from*Pennisetum typhoideum*; isolates from *Digitaria* spp. (including the type for*M. grisea*); and some isolates from *Eleusine* hosts. These conclusions were confirmed by analysis of beta-tubulin and calmodulin sequences which gave the same results but with varying levels of resolution. The only exception was translation elongation factor alpha which did not resolve the *Gaeumannomyces* group from *M. oryzae*. The evolutionary diversification of *M. grisea* appears to be recent and rapid, and the multi-continental distribution of several forms likely reflects human-aided dispersal of infested seed stocks. Clustering of the type species for the genus, *M. salvinii*, with *Gaeumannomyces* rather than with *M. oryzae* and *M. grisea* increases the taxonomic confusion surrounding this genus.

456. A population genetic study of the *Ustilago maydis* virus, a dsRNA killer virus associated with *Ustilago maydis*. Voth, Peter and May, Georgiana. Dept. of Plant Biological Sciences, University of Minnesota, St. Paul, MN USA

Ustilago maydis (corn smut) infects vegetative and reproductive tissues of maize and the teosintes. Associated with *U. maydis* is the *Ustilago maydis* virus (UMV), a double-stranded RNA virus that causes the fungus to produce a "killer" toxin. The proteinaceous UMV toxin is lethal to susceptible *U. maydis* individuals and can inhibit mating between compatible *U. maydis* individuals. UMV is only transmitted through cytoplasmic fusion during mating of compatible *U. maydis* individuals. *U. maydis* individuals are protected from the virus through nuclear resistance or cytoplasmic immunity. The interactions between UMV and *U. maydis* may affect the reproductive biology of *U. maydis*, by enforcing assortative mating in this obligately sexual organism, and subsequently the population genetic structure of UMV. The aim of the current study is to characterize the population genetic structure of UMV throughout the Western Hemisphere through sequence analysis of the viral genome. Using immuno-capture RT-PCR, I have amplified a 500 bp region of the viral capsid protein in isolates from the United States, Mexico, and South America. Sequence analysis of this region revealed nucleotide sequence polymorphisms associated with large scale geographic distance.

457. Population biology of mutations conferring resistance to QoI fungicides in *Pyricularia grisea*. Yun-Sik Kim, Paul Vincelli and Mark Farman, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546, USA.

Strobilurin-based (QoI) fungicides are used to control gray leaf spot disease of perennial ryegrass caused by *Pyricularia grisea*. QoI fungicides bind to cytochrome b, thereby poisoning the cytochrome bc1 respiratory complex. In 2000, QoI-resistant *P. grisea*isolates were identified in Kentucky and Illinois. Characterization of the mitochondrial cytochrome b gene in these cultures revealed two nucleotide substitutions associated with resistance. These cause F129L and G143A substitutions in the cytochrome b protein. Here, we describe experiments to investigate the population biology of QoI resistance in *P. grisea*. The cytochrome b gene was amplified from more than 200 isolates collected in2001and 2002 from perennial ryegrass throughout the eastern and central US. Cleavable Amplified Polymorphic Sequence (CAPS) analysis revealed cytochrome b mutations in isolates from Illinois, Indiana, Kentucky, Maryland and Virginia. Mutant isolates possessed the F129L or G143A mutation but never both. Comparison of sporulation and disease severity revealed no significant differences between mutants and wild-types. A field experiment was conducted to determine if a QoI-resistant population can be superceded by sensitive one(s) after fungicide-mediated selection is lifted. Two years after the last application of a QoI fungicide, we were able to recover only resistant isolates, with a vast predominance of the G143A*CYTB* allele. However, the following year, we recovered significant numbers of F129L mutants and wild-type isolates. We interpret this

population shift to mean that than the latter isolates are more fit than G143A mutants, at least under the conditions present in our field experiment.

458. Organization of genetic variation and relaxed concerted evolution in individuals of arbuscular mycorrhizal fungi. Teresa E. Pawlowska and John W. Taylor. University of California, Plant & Microbial Biology, Berkeley, CA 94720, USA.

Arbuscular mycorrhizal (AM) fungi (Glomeromycota) form symbioses with 85% of terrestrial plants, including many crop species. These obligate biotrophs facilitate plant mineral nutrition and impart to their host tolerance to pathogens and abiotic stress. Coenocytic mycelium and laden with hundreds of nuclei spores of AM fungi yield no morphological evidence of sexual reproduction. Polymorphism of rDNA arrays observed within individual spores inspired a speculation that diverse genomes distributed among different nuclei are maintained and propagated throughout the AM fungal life history. Using a population genetics approach to study transmission of a protein-coding polymorphic genetic marker in single-spore cultures of *Glomus etunicatum,* and PCR-amplification of rDNA variants from individually microdissected nuclei, we demonstrated that nuclei populating hyphae and spores of AM fungi are genetically uniform. Intrasporal rDNA polymorphism is contained within individual nuclei and evinces a relaxation of concerted evolution, a recombination-driven process responsible for homogenization of rDNA arrays dispersed within a genome. Polyploid organization of AM fungal genomes, indicated by our data, could ameliorate deleterious mutations that cannot be purged by recombination in these presumably asexual fungi. Our insights into the organization of genetic variation within glomeromycotan individuals provide foundation for the developmental and population genetics of AM fungi.

459. Heterozygosity in Chytrids. Yajuan Liu, Matt Hodson and Benjamin Hall Biology Department, University of Washington, Seattle, Washington

Chytrids (Chytridiomycota) are the only fungi that , at one stage in the life cycle, produce motile reproductive cells with a single and posteriorly directed flagellum. Molecular phylogenetic studies based on rDNA, RPB1 and RPB2 sequences show that chytrids are the basal lineage of fungi. In our studies of the RPB1 and RPB2 genes from representatives of four fungal phyla, extremely divergent haplotypes of RPB1 and RPB2 are found only in chytrids. Exceptions to this are *Coelomomyces stegomyiae* and *Monoblepharis macraudra* where in each case only one RPB1 and one RPB2 sequence has been found. Each of these organisms is known to have a sexual stage and meiosis in its life cycle. In contrast, two highly divergent haplotypes of RPB1 and RPB2 have been found in *Neocallimastix frontallis* and *Chytridium confervae*, organisms with neither a sexual stage nor meiosis and in Chytriomyces sp. 345D, for which zygotes have been observed but meiosis is not confirmed. For example, the RPB2 haplotypes have 0.2% amino acid and 2.5% nucleotide divergence in Chytridium and are 3.8% amino acid and 5.5% nucleotide divergence in Neocallimastix. This findings suggest that, free from the homogenizing influence of meiotic recombination and allelic gene conversion because they have existed for millions of years without sexual reproduction, in these latter chytrids formerly allelic pairs of genes have diverged extensively, as a result of random independent mutations.

460. Genetic differentiation and phylogeographic structure of *Fusarium graminearum* in Canada. P.K. Mishra[1], J.P. Tewari[1], R.M. Clear[2], and T.K. Turkington[3]. [1]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5; [2]Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main Street, Winnipeg, MB, R3C 3G8; and [3]Lacombe Research Centre, Agriculture and Agri-Food Canada, 6000 C & E Trail, Lacombe, AB, T4L 1W1, Canada.

The fungus *Fusarium graminearum* is a serious pathogen of a range of crop plants in many parts of the world inflicting significant losses. It is also known to produce estrogenic and carcinogenic mycotoxins, which pose an acute risk to human and animal health. We have analyzed 341 isolates of *F. graminearum* collected from different provinces of Canada over many years using restriction digestion of polymerase chain reaction amplified nuclear ribosomal DNA intergenic spacer region (IGS) and inter simple sequence repeats (ISSR) analysis. A substantial genetic diversity was found within the populations. This study will allow deducing various dispersal mechanisms of *F. graminearum* in Canada.

Other Abstracts

461. Possibilities of the maintenance of Shaggy Mane (*Coprinus comatus*), spawn production and growing. Tamás Szabó, József Szarvas, VHajdú Csaba and Nikolett Tóth. Korona Spawn Plant and Strain Researching Laboratory, 3395 Demjén, POB. 1., Hungary. VPh.D. students at Szent István University, School of Horticulture, Budapest, Hungary

The growing of *Coprinus comatus* not so widespread as that of *Agaricus bisporus* and the hybrids of *Pleurotus ostreatus*, *Lentinus edodes*. In Hungary few of the growers is engaged in the growing of mushrooms with curative power. We made collections from more sites of the country from which we created a stock culture (culture in test tubes, cryopreservation, maintenance of the stock on compost). We examined the growth of isolates on 10 different substrate types among laboratory circumstances. We used four types of grain substrate for the creation of a mother spawn, on which we examined the intensity of growth and the possible anomalous mycelium improvement. Arising from the mother spawn we used different substrate mixtures for our inoculatum creational experimentations. Next we created spawn producing then growing experimentations on five substrates for each stock. We examined their morphologies, their changes because of environmental factors, perishability.

462. Differentiation of newly breeded Agaricus hybrids with RAPD method. József Szarvas, Tamás Szabó and Csaba Hajdú, Korona Spawn Plant and Strain Researching Laboratory, 3395 Demjén, POB. 1., Hungary, Ph.D. students at Szent István University, School of Horticulture, Budapest, Hungary

On a world scale, but first of all in Europe and North America a white button mushroom, *Agaricus bisporus* is grown at the highest quantity. Differentiation of champignon species on the basis of traditional morphology and physiology is extremely hard. The appearance of the fruiting body is determined not only by own genetic substance, but by numerous environmental factors as well. Such factors are CO2 level, humidity, temperature and the temperature grade during the yielding period. A strain, which can be marked as a medium hybrid reacts to changes of the environmental factors besides general growing conditions with great versatility, so a medium hybrid can grow as a fresh market hybrid with huge fruiting body or as mushroom to be canned with small fruiting body, depending on the growing circumstances. Starting from the previous facts we would like to differentiate our species – instead of the morphological, perhaps physiological markers, which make the differentiation uncertain – with the help of molecular markers. We used RAPD method for our work, with which - with the use of adequate primers – as a result of the sublimation work the item of difference between our potential strains and primary materials for breeding (431 cultures) can be realized. Through the sequencing of the differentiating bands we synthetized own primers. Our further aims for the future are to be able to detect clones deriving from the cross – inoculation of our own breaded species, so that we can make fingerprints from every of our species with the determined primers. Furthermore we would like to make a selection of homocaryon monospore cultures for the facilitation of breeding work.

463. Characterisation of endopolygalacturonases from *Botrytis cinerea*. Geja Krooshof[1], Harry Kester[1], Ilona Kars[2], Jan van Kan[2], and Jac Benen[1]. [1]Microbiology/Fungal genomics,[2] Phytopathology, Wageningen University, Dreijenlaan 2, 6703 HA Wageningen, The Netherlands

Botrytis cinerea is a plant pathogenic fungus causing serious diseases in a range of economically important crops, such as grapevine, strawberry, kiwi, rose, and flower bulbs. An essential step in the infection process of the fungus is the degradation of the plant cell wall. Therefore, *Botrytis cinerea* secretes a considerable number of cell wall-degrading enzymes, among which are six endopolygalacturonases (BcPGs). The aim of our research is to characterise all six endoPGs in more detail. The methylotrophic yeast *Pichia pastoris*was used to produce the individual BcPGs. Secretion of active recombinant BcPG1, 2, 3, 4, and 6 has been confirmed. Results on the purification and characterization of the BcPG isozymes will be presented.

464. The effect of mismatch repair knockouts on recombination in Neurospora. L. Y. Koh, P. J. Yeadon and D. E. A. Catcheside. School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, South Australia.

DNA mismatch repair genes, *msh-2* and *pms-1*, the eukaryotic homologues of bacterial *MutS* and *MutL*, function in the long patch mismatch repair pathway. These two genes are involved in the correction of errors from DNA replication and the repair of mismatches in heteroduplex DNA formed during genetic recombination. Knockouts of *msh-2* and *pms-1* were generated in Neurospora using RIP. Recombination at *his-3* was measured in three types of cross; 1) homozygous wild-type, 2) heterozygous *msh-2* and 3) homozygous *msh-2*. Additionally, sequence heterology in each of the diploids allowed identification of the parental origin of each section of a conversion tract. In the *msh-2* homozygotes, there was a 2 to 3-fold increase in recombination and no decrease in the frequency of interruptions to conversion tracts.

465. The Functions of a G-alpha and a G-beta Protein in the Life Cycle of the Oomycete *Phytophthora Infestans.* Maita Latijnhouwers, Wubei Dong and Francine Govers Laboratory of Phytopathology, Wageningen University, The Netherlands

The G-protein mediated signaling pathway is a ubiquitous eukaryotic signaling pathway involved in translation of extracellular signals into intracellular ones. Previous work has shown that G-protein mediated signal transduction in plant-pathogenic fungi is indispensable for effcient adaptation to the plant environment and for the development of structures that are required for 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*) subunits, with up to 42% and 62% identity with known G-protein subunits, respectively. Expression studies showed that both genes are differentially expressed in various stages of the life cycle. By means of homology-dependent gene silencing we obtained *P. infestans* transformants deficient in either of the two subunits. Those in which *Pigpb1* is silenced show a defect in asexual sporulation and produce a dense mat of aerial mycelium. Silencing of *Pigpa1* has a major effect on zoospore behavior. Zoospores of *Pigpa1*-silenced mutants show aberrant swimming patterns and fail to autoaggregate. The virulence of these mutants is severely impaired. To isolate downstream targets of PiGPA1, a comparison of gene expression in sporangia of wildtype and of *Pigpa1*-silenced mutants using cDNA-AFLP is in progress. In addition, to elucidate targets of PiGPA1 and PiGPB1, large scale expression profiling of wildtype silenced mutants will be performed in the near future.

466. Investigations into the fungal-fungal interaction between *Verticillium fungicola* and*Agaricus bisporus*. C. Burns[1], R. Amey[1], A. Bailey[1], J. Bowers[1], M.P. Challen[2], T.J. Elliott[2], P.R. Mills[2] and G.D. Foster[1]. [1]School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK [2] Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

Agaricus bisporus (the white button mushroom) accounts for 10% of total horticultural production in the UK. Retail value is £300M in the UK, with a global value of £3,000M p.a. Pathogenic threats to *Agaricus* are varied, with the most significant to the commercial industry being *Verticillium fungicola. Verticillium* infection can occur throughout mushroom development, drastically reducing crop yields. We have used *Agrobacterium* and T-DNA to introduce disruption constructs into *V. fungicola*as part of a molecular investigation into the fungal-fungal interaction between *Verticillium* and *A. bisporus*. We have developed an efficient transformation system for *Verticillium* which we have now adapted to give high levels of targeted mutagenesis. We have used this technology to investigate genes involved in *Verticillium* pathogenicity. We have also developed T-DNA tagging technology in a mycological context for random mutagenesis in *V. fungicola*. We will also report our results in using *Agrobacterium* to transform *Agaricus*, and the range of promoters tested and developed as part of our *Agaricus* Molecular Toolkit which will also allow us to investigate gene expression within *Agaricus*.

467. Transformation of enzyme-treated *Rhizopus oryzae* germlings by square-wave electroporation. Gyan Rai, John N. Tonukari, John P. Rayapati and Christopher Skory. Fermentation Research, Archer Daniels Midland Company and USDA.

Transformation involves the introduction of foreign DNA molecule into a cell for investigating gene structure and function. While bacterial transformations are relatively simple, eukaryotic transformations are always associated with problems that differ greatly between the transforming organisms. This necessitates optimization of complex variables, thereby resulting in a wide array of published transformation protocols available for eukaryotes. These transformation protocols can vary significantly from one species to another, although they can be usually adaptable to organisms within a species. In fungi, the spheroplasts generated from mycelia or spores are routinely transformed by chemical methods or by electroporation. Protoplasts have also been successfully transformed, but this procedure requires tedious optimization and is quite laborious. In some fungal species, intact spores have also been transformed with foreign DNA molecules, albeit with limited success and efficiencies. The biolistics transformation method has proved very helpful in species, including fungi, that have been recalcitrant to the convetional means. The use of this microprojectile bombartment method has been limited because of its cost. We report here a transformation procedure for the partially digested germlings of the filamentous fungus, Rhizopus oryzae. This protocol is relatively simple and time efficient. An overnight culture of spores was washed and further incubated for 2-4 hours with a mixture of lysing enzymes containing chitinase, chitosanase and zymolyase. The mixture of partially digested germlings and DNA was electroporated in a BTX square-wave electroporator. The transforming DNA consisted of a circular plasmid with a functional lactate dehydrogenase and the pyrG genes under control of their respective promoter and terminator sequences. The transformed cells lacked the functional pyrG gene thus allowing selection by functional complementation on minimal plates lacking uracil.

468. Cytological karyotyping of somatic chromosomes from *Phytophthora infestans*, *Mycosphaerella graminicola*, and *Fusarium* spp. Masatoki Taga[1], Cees Waalwijk[2], Wilbert G. Flier[2], and Gert H. J. Kema[2]. [1]Department of Biology, Okayama University, Okayama, Japan. [2]Plant Research International B.V., Wageningen, The Netherlands.

Cytological karyotypes of somatic cells in the ascomycetes, *M. graminicola* and *Fusarium* spp. (*F. graminearum*, *F. pseudograminearum*, *F. culmorum*, *F. cerealis*, and *F. lunulosporum* in the section Discolor) as well as an oomycete, *P. infestans*, were analyzed with the germ tube burst method. We found that *M. graminicola* and *Fusarium* spp. represent the two extremes of cytological karyotypes in filamentous fungi; *M.graminicola* had a large chromosome number (*ca*. 20) with many minute chromosomes (12 chromosomes in a standard strain, < 1 micrometer in length or < 2 Mb in DNA size), whereas only four large chromosomes constituted the genome in the *Fusarium* spp. In *P. infestans*, chromosomes had a previously unknown coiled structure. Most chromosomes were very long, heavily twisted and coiled before their condensation reached the peak, and had a segmented appearance in the highly condensed state. Somatic chromosome counts in *P. infestans* are under way. The results of this study show that cytological karyotyping with somatic nuclei can be practiced in various fungi as well as oomycetes, providing basic information on the genome of each species.

469. Development of Real-time PCR assays for the detection of stem and leaf rust pathogens of wheat. Les J. Szabo. U.S. Department of Agriculture, Agricultural Research Service, Cereal Disease Laboratory, St. Paul, Minnesota, U.S.A.

Historically, stem and leaf rusts have been the one of the most devastating pathogens of wheat, with written accounts of epidemics dating back to ancient Greek and Romans. In North America, major epidemics have occurred from the late 1800's through the 1970's. During the cold war, the United States and the Soviet Union studied *Puccinia graminis* (wheat stem rust pathogen) as a biological warfare agent. Based on the current concern for biosecurity of agricultural crops we are developing Real-time PCR assays for the rapid detection of *P. graminis*, *P. recondita*, *P. triticina* and *P. striiformis* using a TaqMan™ assay system. Current results on the development of Real-time PCR assays for these wheat pathogens will be presented.

470. Somatic cytogenetis in *Neurospora crassa*. Yuya Kato[1], Chizu Ishii[2], and Masatoki Taga[1]. [1]Department of Biology, Okayama University, Okayama, Japan. [2]Department of Regulation Biology, Saitama University, Saitama, Japan.

In *N. crassa*, cytogenetic analysis has been mostly done on meiosis in ascus. Although mitosis in somatic cell or hyphae is a potential target for cytogenetics, the lack of appropriate method to make preparations has hampered the development of somatic cytogenetics of this fungus. In this study, we developed the cytological methods for karyotyping as well as fluorescence *in situ* hybridization (FISH) with germ tube cells of macroconidia. Chromosome specimens were prepared by the germ tube burst method (GTBM; Taga *et al.*, 1998) combining with thiabendazole treatment to accumulate metaphase cells. In the DAPI-stained samples, seven condensed chromosomes were easily distinguished based on their size and constrictions. In addition, difference in chromosome size was detected between a wild type strain and reciprocal translocation mutants. FISH using cosmid clones located near the centromere and telomere of

LGI, rDNA, and beta-tubulin gene as probes was performed onto the interphase and metaphase specimens prepared by GTBM. Fluorescent signals were detected at the expected position for each probe. Thus, somatic cytogentics was shown to be feasible for *N. crassa* in this study.

471. Recombination in Neurospora declines exponentially with distance from *cog*, even across heterologous sequences. P. J. Yeadon, L. Y. Koh, F. J. Bowring, J. P. Rasmussen and D. E. A. Catcheside. School of Biological Sciences, Flinders University, PO Box 2100, Adelaide, South Australia

We have constructed a set of Neurospora strains in which the distance between the recombination initiation site, *cog*, and *his-3*, at which recombination is selected, varies from 1.7 to nearly 6 kb. To maximise recombination, each of the manipulated strains has the more active recombination hotspot allele, cog^L, and the recessive allele of *rec-2*, the unlinked gene controlling local meiotic recombination. In addition each is a histidine mutant, either K26 or K480. We have used these strains to show that the logarithm of the recombination frequency is inversely proportional to the distance from*his-3* to *cog*, a relationship that holds even when the sequences between *his-3* and *cog* are non-homologous.

472. Scanning electron microscopy of fungal mitotic chromosomes and nuclei. Dai Tsuchiya[1] , Hironori Koga [2] , and Masatoki Taga [1]. [1] Department of Biology, Okayama University, Okayama, Japan. [2] Researach Institute of Agricultural Resources, Ishikawa Agricultural College, Ishikawa, Japan.

Fungal chromosomes and nuclei have never been observed by scanning electron microscopy (SEM). In this study, we established the techniques for SEM observation of mitotic chromosomes and nuclei of the ascomycetes of *Cochliobolus heterostrophus* and *Neurospora crassa* , and revealed their ultrastructure at the resolution level of chromatin fiber. The nuclei of various mitotic stages were spread on slide glasses by the germ tube burst method. They were impregnated with osmium and tannin for conductive staining, and freeze-dried using *t* -butyl alcohol. The specimens coated with platinum were observed by field-emission SEM at 20 kV. Metaphase chromosomes were composed of highly condensed chromatin and their overall shape was cylindrical. Apparent constriction indicative of centromere region was found in most chromosomes. Interphase nuclei were more or less spherical, in which 30- nm chromatin fibers were discernible. Each nucleus had a large cavity probably representing the territory of nucleolus. The results from further observation will be presented.

473. Isolation and characterization of DNA polymerase mutants, which are involved in DNA repair and mutagenesis of *Neurospora crassa*. W. Sakai[1], Y. Matsushita[2], Y. Wada[1], Y. Naoi[1], C. Ishii[1], H. Inoue[1]. [1]Saitama Univ., [2]Toyo Univ., JAPAN

In previous meeting, we reported that the *N. crassa upr-1* gene is homolog of the yeast *REV3*. To understand the mechanisms of mutagenic DNA repair in the *N. crassa*more extensively, we identified *N. crassa* homologs of the yeast *REV1* and *REV7* and obtained mutants *ncrev1* and *ncrev7*, which have similar phenotypes to the *upr-1* mutant. *ncrev7* mutant was more sensitive to UV and 4NQO than the wild type. The sensitivity to UV and MMS of the *ncrev1* mutant was moderately higher than that of the wild type, but the sensitivity to 4NQO of the mutant was

similar to that of the wild type. In reversion assay using testers with base substitution or frameshift mutation at the *ad-3A* locus, all *ncrev* mutants showed lower induced-mutability than the wild type. Expression of *ncrevs* was found to be UV-inducible. All *ncrevs* mutants have a normal CPD photolyase gene, however, they showed a partial photoreactivation defect (PPD) phenotype. Furthermore, we characterized UV-induced *ad-3A* mutation-spectrum analysis at the *upr-1* mutant with/without photoreactivation (PR). Most of *ad-3A* mutations at the *upr-1* mutant with PR after UV irradiation are large deletion. These results imply that UPR1 product has an important role for translesion synthesis of non-photoreactable UV lesions.

474. Metabolic activity in filamentous fungi can be analysed by flow cytometry. J. Ron Bradner[1], Paul V. Attfield[2], and K. M. Helena Nevalainen[1]. [1] Department of Biological Sciences, Macquarie University, Sydney NSW 2109, Australia. [2] MicroBiogen Pty Ltd., c/-Department of Biological Sciences, Macquarie University, Sydney NSW 2109, Australia

The use of flow cytometry in combination with fluorescent dyes as a technique to rapidly differentiate and isolate bacterial and yeast cells is well established. We have shown that through the judicial choice of stains the non-destructive screening and sorting of filamentous fungal material is possible. The early stages of growth, from germination through hyphal development of three filamentous fungal species, *Penicillium, Phoma* and *Trichoderma* have been followed using forward and side angle scatter on a Becton Dickenson FACSCalibur flow cytometer. By staining these isolates with the permeant fluorogenic substrates, dihydroethidium and hexidium iodide metabolic activity in the developing hyphae has been measured. We have been able to demonstrate that there is a 12 -13 hour window of opportunity during which germination and the early stages of hyphal development of filamentous fungi can be analysed by flow cytometry. An important feature of flow cytometry is its ability to analyse individually thousands of cells per second. This ability to examine and select from such a vast number of events has the potential to significantly improve the chances of identifying particular fungal mutants and transformants within a much shortened time frame by obviating the need to undergo exhaustive screening on selection medium which is the current practice. The incorporation of genes carrying fluorogenic selection markers, such as green fluorescent protein (GFP) or its analogues, with the target gene construct could provide a convenient avenue for identifying genetically engineered strains.

475. Exploring the potential of HEX1 as a vehicle for foreign protein fusion expression in *Trichoderma reesei.* V. S. Junior Te'o [1], Natalie Curach [1], Peter L. Bergquist[1,2] and K. M. Helena Nevalainen[1]. [1] Department of Biological Sciences, Macquarie University, Sydney NSW 2109, Australia. [2]Department of Molecular Medicine & Pathology, University of Auckland Medical School, Auckland, New Zealand

The search for strong promoters that function under specific conditions, are required for effective gene expression of industrially important gene products. We are currently investigating the use of the catabolite repression insensitive *hex1* gene promoter as an alternative to inducible genes promoters in *T. reesei* (1). A 4376 bp DNA fragment that contains the*hex1* gene open reading frame (ORF) as well as promoter and terminator was isolated from *T. reesei* using Genomic Walking PCR (2). When translated, the 784 bp *hex1* gene ORF gives a peptide sequence of 225 amino acids with an expected molecular mass of 25,207. HEX1 isolated from cell envelope fraction of *T. reesei*, is expressed in different iso-forms also seen on 2-D gels as described in Lim

et al., (3). In this report, we discuss the different iso-forms and demonstrate the potential of HEX1 protein as a carrier in foreign protein expression in filamentous fungi such as *T. reesei*. (1) Curach N, Te'o VJS, Bergquist PL, and Nevalainen KMH (2002). *Hex1*, a new promoter for gene expression in *Trichoderma reesei*. Abstracts of the 6th European Conference on Fungal Genetics. Abstract IIo5. (2) Morris DD, Gibbs MD, Chin CW, Koh MH, Wong KK, Allison RW, Nelson PJ and Bergquist PL (1998). Cloning of the *xynB* gene from *Dictyoglomus thermophilum* Rt46B.1 and action of the gene product on kraft pulp. Appl Environ Microbiol 64:1759-1765. (3) Lim D, Hains P, Walsh B, Bergquist P, and Nevalainen H (2001). Proteins associated with the cell envelope of *Trichoderma reesei*: A proteomic approach. Proteomics 1:899-910.

476. Characterization of the *Neurospora* homologue of the human gene *nbs1*. Yukiko Kikuchi, Akihiro Kato, Hirokazu Inoue. Laboratory of Genetics, Department of Regulation Biology, Faculty of Science, Saitama University, Saitama City 338-8570, Japan.

Double-strand break is induced by ionizing radiation and also occur during DNA replication. There are at least two pathways which can repair such breaks. They are non-homologous end joining (NHEJ) and homologous recombination (HR). *nbs1* gene in human, which is a responsible gene of Nijmegen breakage syndrome, recruits a hMRE11/hRAD50 complex to sites of DSBs. NBS1/hMRE11/hRAD50 complex plays roles in processing DNA ends, selecting either NHEJ or HR and in activation of cell cycle checkpoint. A newly isolated mutant defective in the *Neurospora* homologue of the human NBS1 was found to be highly sensitive to a wide variety of mutagenes, including UV light, methyl methanesulfonate (MMS), 4-nitroquinoline 1-oxide (4NQO),*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NG), tert-butyl hydroperoxide (TBHP), hydroxy urea (HU) and ethyl methanesulfonate (EMS). This mutant was sensitive to histidine (HIS) and camptothecin (CPT), too. The *ncNBS1* gene is located on the right arm of LGVII between *met-7* and *arg-10*. We investigated epistatic relationships between *ncNBS1* and other repair genes. Further characterization of the mutant will be presented.

477. Progress in Transforming the Mushroom *Agaricus bisporus*: Agrobacterium Methodologies and the Development of Novel Marker Genes. K. A. Leach[1, 2]; M. P. Challen[1], T. J. Elliott1 and J. Henderson[2]. [1]Horticulture Research International, Wellesbourne, Warwick, UK, CV35 9EF. [2]Coventry University, School of Science and the Environment, Priory Street, Coventry, UK, CV1 5FB

Successful transformation of the button mushroom *Agaricus bisporus* has recently become possible due to the application of Agrobacterium-mediated transformation. Factors such as the strain of *Agrobacterium tumefaciens*, co-cultivation conditions, host strain, type of tissue, and the use of various promoters are known to affect rates of transformation. Using gill tissue from a number of commercial A. bisporus strains we have shown that different Agrobacterium strains and the use of vacuum infiltration and sonication treatments can dramatically alter the recovery of transformants. The effect of the virulence inducing phenolic, acetosyringone, on the number of transgene copies integrated into the *A. bisporus* genome has also been investigated. The rate of gill tissue transformation to hygromycin resistance was highest using *A. tumefaciens* strain AGL-1 and a sonication treatment (ca. 70 – 99% efficient). There is a need for alternative selectable markers in mushroom transformation. Homobasidiomycete para-aminobenzoic acid genes can confer sulfonamide resistance and have potential as positively selectable markers. The

Coprinus cinereus auxotroph, PG78, was transformed to PAB+ prototrophy using PEG-mediated protoplast transformation and the C. cinereus pabA gene. PAB+ prototrophs were also generated using heterologous *C. bilanatus* and *A. bitorquis* pab genes. Most PAB+ transformants have proved resistant to sulphanilamide and sulfamethoxazole. Southern blotting is normally used to confirm transgene copy number. With PAB+ transformants we have demonstrated the use of real-time quantitative PCR to determine copy number.

478. Direct cloning of fungicide resistance genes in *Botrytis cinerea*. Sabine Fillinger, Catherine Lanen and Pierre Leroux Unite de Phytopharmacie & Mediateurs Chimiques, INRA, Versailles, France

Botrytis cinerea is the causal agent of grey mould on fruits, vegetables, ornamentals... Chemical control remains the principal means to reduce the incidence of grey mould on these crops and especially on grapevine, but rapid development of resistant strains involves development of anti-resistance strategies (Leroux et al., 2002). Theses strategies require the understanding of the resistance mechanisms in *B. cinerea* mutants. Direct cloning of these mutations involves transformation frequencies higher than spontaneous mutation and tagging of the resistance marker for its identification. The autonomously replicating plasmid pFAC1 harboring human telomeric sequences and the hygromycin resistance marker (Javerzat et al., 1993) transforms *B. cinerea* with frequencies up to 20000 transformants/ug DNA. Combining linearized pFAC1 and restricted genomic DNA for transformation and selecting for the double resistance, to hygromycin and to the fungicide to be analyzed, makes it possible to clone the fungicide resistance gene in *B. cinerea*. The pFAC1 derivative remains linear in the transformant in the presence of hygromycin and/or the fungicide. The insert can be characterized after PCR-amplification. We will present results obtained with DNA from field-isolates resistant to anilinopyrimidine fungicides. Javerzat, J. P., Bhattacherjee, V., and Barreau, C. (1993). Nucleic Acids Res 21, 497-504. Leroux, P. et al. (2002). Pest Managment Sci 58(9),876-88.

479. Molecular analysis of the multiallelic B mating locus that encodes pheromone and receptors in *Coprinus cinereus*. Meritxell Riquelme[1], Andrew J. Brown[2] and Lorna A. Casselton[1]. [1]Department of Plant Sciences, University of Oxford, Oxford, OX1 3RB, UK. [2]Molecular Pharmacology, GlaxoSmithKline, Stevenage, SG1 2NY, UK

Pheromone signaling plays an essential role in fungal mating. In the homobasidiomycete*Coprinus cinereus*, the mating pheromones and receptors have been recruited for self-nonself recognition and are encoded by multiallelic genes at the B mating type locus. The pheromone receptors belong to the rhodopsine-like superfamily of 7-transmembrane domain G-protein coupled-receptors (GPCRs) and specifically to the *S. cerevisiae* MATa cell receptor Ste3p subfamily. The lipopeptidic pheromone precursors are processed and secreted in the same manner as the *S. cerevisiae* a-factor. Rhodopsin-like GPCRs have evolved different specificities to respond to a variety of ligands. In fungi, and specifically in *C. cinereus*, a similar evolutionary process has created a family of functionally redundant receptors and corresponding ligands. Sequence variation permits them to display highly specific interactions: a single receptor may be activated by several different pheromones and a single pheromone may activate several different receptors. The different versions of the B locus derive from different allelic combinations of three sets of paralogous genes termed groups 1, 2, and 3. We have isolated genes from 18 strains

in our collection with different B mating specificities. Cross-hybridisation in Southern blots and PCR amplification has been used to identify homoalleles of a particular subfamily of genes in these different B loci. Long range PCR has allowed us to amplify other new alleles. Thus far, we have identified 5, 6, and 2 alleles in groups 3, 2 and 1, respectively. We predict the existence of at least 4 more new alleles: one for group 1 and two or three for group 3. The family of *C. cinereus* receptors and pheromones offers a new dimension in studies of GPCRs, which are currently the largest protein family of validated drug targets. Our analysis of these different allelic versions of pheromone and receptors in wild type strains of *C. cinereus* is allowing us to predict the structure-function relationship determinants of ligand specificity. In addition, molecular genetic analyses of the B locus in *C. cinereus* provide clues toward understanding the evolution of so many different specificities of the B-mating type.

480. Molecular analysis of breeding behaviour in Agaricus species. Y. Li*, M. P. Challen++, T. Elliott++ and L. A. Casselton*. *University of Oxford, Department of Plant Sciences, Oxford, OX1 3RB, UK ++Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

Agaricus bisporus, the cultivated mushroom, has a single mating type locus that determines mating compatibility. As the name implies, only two spores are produced following meiosis. Partitioning of haploid nuclei of opposite mating type into each basidiospore means that each germinates to give a fertile heterokaryon. This breeding behaviour is known as secondarily homothallic. Wild isolates of this species may have a more conventional heterothallic breeding behaviour in which the meiotic nuclei are segregated into four different spores. These spores are self-sterile and develop as homokaryons that must fuse with a compatible partner in order to generate a fertile heterokaryon. Other wild members of the genus may be truly self fertile and no longer need to mate to produce fruit bodies. Our aim is to clone the mating type genes of the cultivated *A. bisporus* and its close wild relatives to see if breeding behaviour can be related to mating type gene organisation and function. We have identified a pair of divergently transcribed homeobox genes within the mating type locus of *A. bisporus*. We are currently using PCR strategies to establish how many allelic variants of these genes exist in 15 different strains that exhibit different mating specificities. This analysis should reveal whether a single pair of genes is sufficient to generate the allelic variation displayed in this collection.

481. Identification and mapping of avirulence genes from *Magnaporthe grisea*. Claudia Kaye[1], Joelle Milazzo[1], Yanli Wang[2], Ying Shen[2], Marc-Henri Lebrun[3] and Didier Tharreau[1]. [1]UMR 385, Cirad, Montpellier, France [2]CNRRI, Hangzhou, China [3]UMR1932, CNRS-Bayer Crop Science, Lyon, France

We have begun a study aimed at mapping different *Magnaporthe grisea* avirulence (avr) genes. Crosses were made between fertile isolates and pathology tests were performed to uncover segregating avr genes. From the first cross, three different avr genes were uncovered. Pathology tests for two of these have been completed. The third gene shows a more complicated inheritance implicating possibly two genes. Fine mapping using AFLP was achieved to find genetic markers closely linked to the first two genes. Markers were found on either side of each of the genes. A second part of this project involved the development and mapping of microsatellite or SSR (single sequence repeat) markers. These microsatellite markers can be used to link maps from diverse crosses with a reference map. Several of these markers enabled us to locate the two avr

genes to a specific chromosome and region. A search of the *Magnaporthe grisea* database allowed us to find other microsatellite markers closely linked to a gene on chromosome 7 and therefore approach the gene for eventual cloning. A BAC library has been constructed from DNA isolated from the avirluent parent for all three of the above described genes. The library has been screened with probes made from sequences closely linked to the gene on chromosome 7. Several candidate BACs have been selected for transformation into the virulent parent. Pathology tests will determine if any of these BAC clones contain the gene of interest.

482. A System for Studying Genetic Changes in *Candida albicans* During Infection. A. Forche, G. May, J. Beckerman, S. Kauffman, J. Becker, and P. T. Magee. University of Minnesota. GCD

Candida albicans is a diploid yeast with a dimorphic life history. It exists commensally in many healthy humans but becomes a potent pathogen in immunocompromised hosts. The underlying genetic mechanisms by which *C. albicans* switches from a commensal to a pathogenic form in the host are not well understood. To study the evolution of virulence in mammalian hosts, we used *GAL1* as selectable marker system that allows for both positive and negative selection in selective media. We show that the deletion of one or both copies of *GAL1* in the *Candida albicans* genome does not change virulence in a systemic mouse model. We obtained estimates for the frequency of mitotic recombination at the *GAL1* locus during systemic infection. Our observations suggest that genetic changes such as mitotic recombination and gene conversion occur at a high enough frequency to be important in the transition of *Candida albicans* from a commensal to a pathogenic organism. We have identified 432 SNPs (single nucleotide polymorphisms) in the *GAL1*heterozygote (http://sequence-www.stanford.edu/group/candida/index.html). To study mitotic recombination at the population level, microarrays will be developed to facilitate high-throughput analyses of populations of *Candida albicans*. To analyze mitotic recombination *in vivo*, populations will be analyzed by SNP microarrays prior to and after mouse passage. This work was supported by a NIH grant AI46351 awarded to P. T. Magee and G. May.

483. Evidence for repeat-induced point mutation (RIP) among sequences of a putative transposable element from the wheat pathogen *Mycosphaerella graminicola*. Stephen B. Goodwin[1], Yang Tian[2], and Jessica R. Cavaletto[1]. [1] USDA-ARS, [2] Department of Botany and Plant Pathology, 915 West State Street, Purdue University, West Lafayette, IN 47907-2054, USA

Repeat-induced point mutation (RIP) is a potential mechanism for inactivating transposons in fungi through introduction of C to T transitions into coding regions. This phenomenon has been analyzed thoroughly in *Neurospora*, but is less well studied in other fungi. A DNA fingerprint probe from the septoria tritici leaf blotch pathogen of wheat, *Mycosphaerella graminicola*, was shown previously to contain part of an active transposable element. Transposition of this element appeared to be stimulated by meiosis, but also occurred at a fairly high frequency in one isolate during asexual reproduction. To test for possible RIP in *M. graminicola*, five copies of the reverse-transcriptase gene from the putative transposable element were cloned and sequenced. A high frequency of G:C to A:T transitions characteristic of RIP was found among the sequences of the five clones. These mutations occurred not only in the reading frame for the reverse-

transcriptase gene but also in the presumably non-coding regions of the transposon. All copies except one contained one or more stop codons within the region coding for the reverse transcriptase. Most of these stop-codon mutations were caused by G:C to A:T transitions, as expected if RIP is the causal mechanism. The copy of the transposon without stop codons presumably may be active. By comparison, analysis of the single-copy *Sln1* gene from *M. graminicola* showed no evidence of RIP. This may provide the first evidence for RIP in a Loculoascomycete and indicates that this phenomenon may be important for inactivating transposons in many fungi in the genus *Mycosphaerella* and possibly the order Dothideales.

484. Analysis of sexually compatible *C. lindemuthianum* isolates from Mexico. Raul Rodriguez, Maria-Teresa Ramirez and June Simpson, CINVESTAV, Unidad Irapuato, Mexico

The genus Colletotrichum contains 39 species, many of which are pathogens of economically important plants. Eight of these species undergo sexual reproduction under laboratory conditions and both heterothallic and homothallic sexual forms have been observed. Sexual reproduction in *C. lindemuthianum* the causal agent of anthracnose in common bean is of the heterothallic form, but is rare and difficult to reproduce. Few sexually compatible*C. lindemuthianum* isolates have been described previously. Compatible isolates produce few reproductive structures many of which are inviable. Nineteen Mexican *C. lindemuthianum* isolates were analysed in order to determine their capacity to develop sexually reproductive structures. All isolates were self-sterile, eight confrontations produced only inviable proto-perithecia and one confrontation produced fertile perithecia containing asci and ascospores. All other confrontations were infertile. AFLP analysis of colonies derived from individual ascospores confirmed that they were progeny from a sexual cross. Analysis of 44 individual ascospores mainly from different asci showed 42 distinct genotypes, all of which were combinations of the original parental genotypes. Markers analysed segregated in a proportion of 1:1 confirming that the progeny originated through sexual recombination. Analysis of this segregating population will allow genetic analysis of important traits such as avirulence, pathogenicity and sexual compatibility.

485. Meiotic Silencing in *Neurospora* is Mediated by Specific DNA Elements. Dong Whan Lee, Kye-Yong Seong, Kevin Baker and Rodolfo Aramayo. Department of Biology. College of Science. Texas A&M University. College Station, TX 77843-3258. USA.

Altering the normal ploidy in *Neurospora crassa* activates RNA-mediated gene silencing mechanisms, called "quelling" in haploid cells and "meiotic silencing" in diploid cells. Although these two events share equivalent molecular components, they respond to different signals. Repetitive elements have been postulated to trigger quelling, while unpaired DNA has been postulated to trigger meiotic silencing. This work was aimed at identifying signals, if any, that would be required to be present in the unpaired DNA to trigger meiotic silencing. We started by scanning the chromosomal region corresponding to the Ascospore maturation-1 (*Asm-1*) reporter gene of *Neurospora crassa* (Aramayo et al. (1996). Genetics 144, 991-1003) and found two such regions that we call Meiotic Silencing Inducing RU/u>egions (MSIRs). The first one, located immediately upstream of the coding region can induce meiotic silencing in cis (i.e., silence itself), but not in trans (i.e., silence paired copies of *Asm-1* present elsewhere). The second one, located downstream, is equally capable of inducing silencing both in cis and in trans. Here, we describe some of the properties of these MSIRs. In addition, we demonstrate the size of the

unpaired DNA loop determines the strength of meiotic silencing. Finally, we demonstrate that the signal produced by the unpaired DNA loop does not propagate to adjacent paired genes. These findings are discussed in the context of the biology of meiotic chromosomes. This work was supported by U. S. Public Health Service Grant GM58770 to R. A.

486. Identification of Suppressors of Meiotic Silencing. Dong Whan Lee, Malcolm McLaughlin, Robert J. Pratt, Kevin Baker and Rodolfo Aramayo. Department of Biology. College of Science. Texas A&M University. College Station, TX 77843-3258. USA.

During meiosis, chromosomes 'sense' each other through a process called meiotic transvection (Aramayo, R. and Metzenberg, R. L. (1996), *Cell* **86**, 103-113), which was discovered by studying the complex *Ascospore maturation-1* (*Asm-1*) locus of *Neurospora crassa* (Aramayo *et al.* (1996), *Genetics* **144** , 991-1003). The presence of unpaired DNA was recently proposed to activate RNA silencing (Shiu *et al.* (2001), *Cell***107** , 905-916), based on the demonstration that mutations in an RNA-dependent RNA polymerase (RdRP) gene called *Suppressor of ascus dominance-1* (*Sad-1*), eliminate the ascus-dominance of unpaired DNA from *Asm-1* and other genes (Shiu *et al,* (2001), *Cell* **107** , 905-916). Scanning of the *Neurospora* genome revealed the existence of a paralog for *quelling deficient-2* (*qde-2*) which we call *Suppressor of meiotic silencing-2* (*Sms-2*--NCU009434.1) and of two Dicer-like proteins related to the ribonucleases of the carpel factory, which we call *Suppressor of meiotic silencing-3* (*Sms-3* --NCU08270.1) and *dicer-like-2*(*dcl-2*-- NCU06766.1). In this work; we first demonstrate the involvement of both *Sms-2* and *Sms-3* in meiotic silencing. We next demonstrate that the SAD-1 RNA-dependent RNA polymerase is only essential for amplification, not production, of the meiotic silencing signal if double-stranded RNA corresponding to the silenced gene is produced by an alternative pathway. Finally, we hypothesize that *Sad-1, Sms-2* and *Sms-3* are components of the same meiotic silencing pathway. R. J. P. was partially supported by the Program in Microbial Genetics and Genomics (PMGG). This work was supported by U. S. Public Health Service Grant GM58770 to R. A.

487. A novel B-ZIP transcription factor from the rice blast fungus, *Magnaporthe grisea*, is required for pathogenicity on rice. Andrew Tag [1], Pierre-Henri Clergeot[2], Karine Lambou[2] , Stéphanie Sibuet[3], Christelle Barbisan[3], Marie Pascale Latorse[3], Philippe Perret[3], Roland Beffa[3], Josh Munson[1], Terry Thomas[2], and *Marc-Henri Lebrun[2]. [1]Laboratory for Functional Genomics, Department of Biology, Texas A&M University, College Station, TX USA. [2]CNRS-Bayer CropScience, Lyon, France[3]Bayer CropScience, Lyon, France

Genes required for pathogenicity of *M. grisea* were identified using non-pathogenic mutants obtained by REMI-insertional mutagenesis. We recovered three tagged non-pathogenic mutants (M421/PLS1, M700/BUF1, and M763). The number of lesions caused by mutant M763 was dramatically reduced compared to wild type (-95%), and its colonization of host tissues was also impaired (-80%). Cytological analysis of the penetration process showed that M763 differentiated appressoria at normal rates, but their penetration efficiency was highly reduced. Colonization of epidermal cells occurred normally but the mutant did not efficiently invade underlying leaf tissues. Genomic regions flanking the plasmid insertion were rescued and used to recover corresponding genomic and cDNA clones. Complementation analysis identified an ORF inactivated in M763 that encodes a novel B-ZIP transcription factor from the GCN4 superfamily.

This gene is only expressed in spores and during plant infection and it is likely to control the expression of genes that are required for efficient penetration and colonization of host plant tissues. Cell biology and expression studies are being pursued in order to identify the cellular functions controlled by this gene. In particular, genes that are differentially expressed between wild type and the M763 mutant will be identified using a *M. grisea* EST-microarray.

488. Assessment of basal levels of expression of defense-related genes in soybean cultivars varying in quantitative (partial) resistance to *Phytophthora sojae*. Miguel E. Vega-Sanchez[1], Margaret G. Redinbaugh[2], Anne E. Dorrance[1]. [1]The Ohio State University, Ohio Agricultural Research and Development Center, and [2]USDA Agricultural Research Service, Wooster, OH 44691, USA.

Phytophthora sojae is an oomycete pathogen that causes economically important root and stem rot diseases of soybean. Quantitative or partial resistance in this pathosystem is characterized by containment of the pathogen to the lower stem and tap root, but the genetic and/or biochemical mechanisms underlying the expression of partial resistance to *P. sojae* in soybean are poorly understood. To explore the hypothesis that higher basal expression levels of defense-related genes correlate with quantitative disease resistance, Northern blot analysis of root and cotyledon total RNA from 14 soybean cultivars varying in partial resistance levels to *P. sojae* was carried out. Ranking of cultivars into low, moderate and high partial resistant groups was done based on a standard lesion growth test. The coding sequences of eight defense-related genes including pathogenesis-related (PR) proteins and enzymes of the phenylpropanoid pathway were used to design primers for RT-PCR. The cDNAs representing a portion of the coding regions were cloned into a plasmid vector and used as probes. Preliminary results show that no clear-cut associations exist between constitutive defense-related gene expression levels and quantitative resistance to *P. sojae* in soybean. However, differential basal levels of expression of defense genes was observed between roots and cotyledons: most transcripts were at higher levels in roots than in cotyledons, with some mRNAs (PR1 and basic peroxidase) being undetectable in cotyledons. Two exceptions were matrix metalloproteinase (GmMMP2) and beta-1, 3-endoglucanase which were expressed at higher levels in cotyledons than roots.

489. H_2O_2 resistance of microfungi isolated from regions of various radioactivity. Tatiana A.Belozerskaya[1], Yulia V.Blazheevskaya[2], Konstantin B. Aslanidi[3], Anna E.Ivanova[4], Nelly N. Zhdanova[2]. [1]A.N. Bach Inst. Biochem., RAS, Moscow, Russia, [2]Inst. Microbiol. Virol., NAS, Kyiv, Ukraine, [3]Inst. Theor. Exp. Biophys., RAS, Pushchino, Moscow region, Russia, [4]Soil Science Faculty, Moscow State University, Moscow, Russia

High resistance of microfungi to radiation in comparison with animals, plants and bacteria puts forward the question about their tolerance to oxidative stress caused by ionizing radiation. Video image analysis was used to study growth peculiarities and H_2O_2 resistance of micromycetes isolated from territories of background radiation (K), and from such radioactive substrates as the inner locations of 4th Unit of ChNPP and soils of 10-km limit zone (R). Six strains of microfungi were analyzed: *Alternaria alternata* (K, R), *Cladosporium cladosporoides* (K, R), and *Paecillomyces lilacinus* (K, R). Two different growth strategies were elucidated in the (R) fingi. *A. alternata* (R) and *P. lilacinus* (R) strains showed hyphal aggregation and an increase in initial growth rates. On the other hand, *C.cladosporoides* (R) did not differ significantly from the (K)

strain, but its growth rate was two times lower than that of the (K) one. It turned out that in all (R) strains growth was inhibited at H_2O_2 concentrations of 10^{-2}-10^{-1} M, while in (K) strains - at the concentrations of 10^{-3}-10^{-2} M. The unique strain (*A. alternata* K) demonstrated an increase in growth rate at the H_2O_2 concentrations of 10^{-7}-10^{-5} M. Restoration of growth was observed only in the (R) strains upon transfer to the control medium following incubation under high (10^{-2} M) H_2O_2.

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490. Analysis of a G protein coupled receptor from *Neurospora crassa* with similarity to putative carbon sensory receptors from yeasts. Liande Li and Katherine A. Borkovich. Department of Plant Pathology, University of California, Riverside. Riverside, CA 92521

We have identified a gene encoding a G protein coupled receptor in the *Neurospora* genome database (http://www-genome.wi.mit.edu/annotation/fungi/neurospora) that is most similar to Gpr1p from *Saccharomyces cerevisiae* and Git3 from *Schizosaccharomyces pombe*. The two yeast receptors are coupled to G alpha subunits that modulate cAMP levels in response to carbon availability in these species. The *N. crassa* homologue of these yeast G alpha proteins is GNA-3, for which our laboratory has previously demonstrated roles in regulation of adenylyl cyclase protein levels and conidiation. We have identified cosmids containing the *N. crassa* gene (*gpr-4*; G protein coupled receptor –4), constructed a gene replacement vector and isolated *N. crassa* strains lacking the wild-type *gpr-4* gene. Experiments are in progress to determine the cellular phenotypes of *gpr-4* deletion mutants and to explore coupling to the three *N. crassa* G alpha proteins.

491. Rice blast secondary metabolism signals pathogen attack. H. U. Böhnert, I. Fudal and M.-H. Lebrun. CNRS/Bayer CropScience, Lyon, France.

Rice resistance to the blast fungus *Magnaporthe grisea* depends on specific interactions controlled by fungal avirulence genes and corresponding plant resistance genes. Isolate Guy11 is avirulent towards rice varieties carrying the resistance gene *Pi33.* The corresponding avirulence gene *ACE1* was isolated by positional cloning and was found to encode a fungal type I polyketide synthase (PKS) bearing high similarity to LNKS from *A. terreus* (involved in lovastatin biosynthesis). However, in contrast to LNKS, ACE1 carries a complete NRPS module at the C-terminus. Several lines of evidence, including the molecular analysis of a spontaneous virulent mutant, support the notion that this C-terminal NRPS module is functional.

Using an ACE1-GFP fusion, we demonstrated cytoplasmic localization of the synthase. By introducing a point mutation in the ketosynthase domain we created a nonfunctional allele that is unable to confer avirulence. We conclude that it is the metabolite produced by ACE1 which is recognized by resistant host plants and triggers resistance.

Unlike most fungal PKS-encoding genes studied to date, *ACE1* is not expressed in culture. Instead, *ACE1* expression is restricted to the penetration phase of pathogenic development and can be detected both on plant and artificial surfaces. No expression was detected in the

penetration-deficient mutant buf (impaired in melanin biosynthesis). In *M. grisea*, melanin is required for the build-up of turgor pressure in the appressorium, an essential prerequisite for penetration. Our results suggest that *ACE1* expression requires appressorial turgor and is developmentally regulated.

492. Integrating graduate and undergraduate education. Patricia J. Pukkila. Office of Undergraduate Research, U. North Carolina-Chapel Hill.

On many campuses, the opportunity to carry out an original investigation has become a distinctive feature of the undergraduate experience. The Office of Undergraduate Research at UNC-Chapel Hill seeks to expand these opportunities by eliminating some of the artificial boundaries between graduate and undergraduate education. Elements of our programs that are relevant to teaching fungal biology and genetics include collaborative design and facilitation of undergraduate "inquiry seminars" that focus on the logic of scientific investigation, workshops and course work on mentoring skills, graduate contributions to a database of research opportunities for undergraduates, financial compensation for graduate students to encourage careful design of undergraduate research opportunities and subsequent reflection on the mentoring experience, and graduate involvement in undergraduate presentations at national meetings and for elected officials. In addition to enhancing the undergraduate experience, these programs contribute to graduate professional development and to the public understanding of emerging issues in fungal biology and genetics.

493. Using fungal genomes to teach bioinformatics to undergraduates. Steven W. James. Biology Department & Biochemistry and Molecular Biology Program, Gettysburg College, Gettysburg, PA.

Bioinformatics and functional genomics have, in a few short years, become indispensable tools of the molecular biologist. Putting these powerful tools into the hands of aspiring students provides a rigorous intellectual challenge and fosters original thinking and problem-solving skills necessary for success as an independent investigator. Fungal genomes furnish an ideal training ground for *in silico* gene discovery and analysis, owing to their high gene density and the relative diminution of introns. In the Molecular Genetics course at Gettysburg College, juniors and seniors undertake a six-week project to discover and analyze genes within a 50-100 kb fragment of the *Neurospora crassa* genome. Using a combination of desktop software and web-based tools, pairs of students collaborate on a segment of their own choosing to identify and characterize a minimum of six genes that share homology with known genes from other organisms. Students use DNA Strider 1.0 to identify ORFs and convert them to protein sequences. They perform blastp searches using Genbank (NCBI), and learn how to judge the quality of hits, predict orthologs, and navigate auxiliary Genbank resources for further information about the genes. Students make extensive use of the Saccharomyces Genome Database (SGD), examining expression profiles and protein-protein interactions of the yeast homologs of their genes. The students assemble their findings into a comprehensive report, in which they analyze gene functions and demonstrate a detailed understanding of microarray techniques and proteomic approaches. (Supported by Gettysburg College).

494. Physical mapping of the genome of the fungal pathogen *Ustilago hordei* and characterization of the 500 kb MAT-1 sequence. G. Jiang, G. Bakkeren, R. Warren, Y. Butterfield, R. Chui, J. Schein, H. Shin, N. Lee, D. Kupfer, Y. Tang, B. Roe, S. Jones, M. Marra and J. Kronstad. Biotechnology Laboratory, Department of Microbiology and Immunology, and Faculty of Agricultural Sciences, The University of British Columbia, Vancouver, B.C. V6T 1Z3, Canada. Agriculture and Agri-Food Canada, Summerland B.C., Canada. Genome Sciences Centre, B.C. Cancer Agency, 600 W. 10th Avenue,Vancouver, B.C. V5Z 4E6, Canada. The Advanced Center for Genome Technology, The University of Oklahoma, Norman, Oklahoma 73019-3051

The basidiomycete fungus *Ustilago hordei* causes covered smut disease of barley and oats. In this pathogen, sexual development and the ability to infect the host plant are controlled by the MAT locus with two alternative types MAT-1 and MAT-2. The MAT locus is unusually large compared with other fungal mating loci (~ 500 kb for MAT-1 and ~ 430 kb for MAT-2) and recombination is suppressed between the a and b gene complexes that define the boundaries of the MAT locus. We constructed a physical map of the *U. hordei* genome by fingerprinting a bacterial artificial chromosome (BAC) library; the map consists of 2030 BAC clones that have been assembled into 51 contigs containing approximately 23 Mb of DNA. The physical map provided information on the rDNA repeat structure for *U. hordei* and allowed us to define a 1.76 Mb contig that spans the MAT-1 locus. A set of minimum tiling path clones was identified for this contig and the order of these clones was confirmed by BAC-end sequencing and hybridization. Five BAC clones were identified that provided the minimum tiling path between the a and the b gene complexes. These BACs now have been sequenced and reveal a preponderance of retrotransposon elements as well as several predicted genes with functions not obviously related to mating. The characterization of the MAT-1 locus provides an opportunity to compare the organization of mating-type loci between basidiomycete fungi and explore evolutionary aspects of sex-chromosomes.

495. Disruption of chitin synthases in *Coccidioides posadasii*, the Valley Fever fungus. M. Alejandra Mandel, Ellen M. Kellner, John N. Galgiani and Marc J. Orbach. University of Arizona

Coocidioides immitis and *Coccidioides posadasii* are dimorphic fungal pathogens of humans and other mammals that are endemic to the desert Southwestern US. During infection, the fungus switches from polar hyphal growth and conidial production to isotropic spherule growth and subdivision of spherules into endospores. This process involves major synthesis of new cell wall material. To investigate the role chitin synthases (CHSes) may play in the infectious phase of Coccidioides sp., we have isolated six CHS genes corresponding to individual members in classes I-IV and two members of class V. Data will be presented on their expression patterns during hyphal growth and the infection cycle of C. posadasii. Agrobacterium-mediated transformation of *C. posadasii* has been used to disrupt three of these genes. Phenotypic analyses of the disruptants will be presented.

496. A 3-hydroxyacyl-CoA Dehydrogenase Encoded at the C*ochliobolus heterostrophus* Tox1A Locus is Required for T-toxin Production and High Virulence to T-cytoplasm Corn.

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Cochliobolus heterostrophus race T produces T-toxin, a polyketide required for high virulence of the fungus to Texas male sterile (T)-cytoplasm corn. Ability to produce T-toxin segregates as two unlinked loci, Tox1A on chromosome 12;6 and Tox1B on chromosome 6;12. Previous work has shown that genes at Tox1A (PKS1 and PKS2) and at Tox1B (DEC1) are essential for T-toxin production. A second gene at Tox1B (RED1), is not required. To determine if additional genes are required for T-toxin production, the sequences of the known Tox1 genes were used to query the *C. heterostrophus* genome sequence database (Torrey Mesa Research Institute). Three genes - LAM1 at Tox1A on a contig carrying PKS2, and RED2 and RED3 at Tox1B on a contig carrying DEC1- were recovered. LAM1 (similar to 3-hydroxyacyl-CoA dehydrogenase) was found upstream of PKS2, and RED2 and RED3 (similar to short chain dehydrogenases) were found downstream of RED1. Targeted disruption of LAM1 greatly reduced both T-toxin production and virulence to T-cytoplasm corn, whereas disruption of all three reductase genes RED1, RED2 and RED3 had no apparent effect on T-toxin production or on virulence.

497. Re-annotation of ORF start and stop sites in *S. cerevisiae* based on comparisons of orthologous ORFs from five other *Saccharomyces* species. Christie, K. R.[1], Sethuraman, A.[1], Balakrishnan, R.[1], Dolinski, K.[1], Dwight, S. S.[1], Fisk, D. G.[1], Hong, E. L.[1], Theesfeld, C. L.[1], Kamvysselis, M.[2], Paul Cliften, P.[3], Costanzo, M. C.[1], Engel, S. R.[1], Issel-Tarver, L.[1], Dong, S.[1], Weng, S.[1], Johnston, M.[3], Lander, E.[2], Botstein, D.[1], and Cherry, J. M.[1]. [1] Department of Genetics, Stanford University, Stanford, CA. [2] Whitehead Institute for Biomedical Research, MIT, Cambridge, MA. [3] Department of Genetics, Washington University School of Medicine, St. Louis, MO.

Since the publication of the genome sequence of *S. cerevisiae* in 1996, corrections have been ongoing to improve the accuracy of the sequence and the annotations of open reading frames (ORFs) and other sequence features. While most of these changes have been made on a gene by gene basis, the availability of genome sequences for five *Saccharomyces* species (*S. paradoxus*, *S. mikatae*, *S. bayanus*, *S. kluyveri*, and *S. castelli*) from groups at MIT and Washington University allowed the *Saccharomyces* Genome Database (SGD) to make a large scale comparison of orthologous ORFs in these species and re-evaluate ORF boundaries in *Saccharomyces cerevisiae*. Using the Fungal Alignment viewer and Synteny Viewer (links under Comparison Resources on SGD locus pages), users may view protein and DNA sequence from the other *Saccharomyces* species. For 402 of the ORFs considered, there were differences in the length of the one or more ORFs relative to other species at either or both the amino-terminal and carboxyl-terminal ends of the predicted protein sequence. Careful comparison of the DNA sequences, predicted protein sequences, and available literature for these ORFs has allowed us to conclude that changes should be made to the ORF start and/or stop sites for 104 ORFs. A further 161 changes were suggested, but require additional sequencing of *S. cerevisiae* S288C for confirmation. Any changes made will be documented in Locus History notes and a paper will be published detailing the changes to be made. Thus, comparison of *S. cerevisiae* with three closely related and two more distantly related *Saccharomyces* species has allowed us to improve the ORF annotations in SGD.

498. Characterization of the Glucose-6-phosphate Isomerase Gene in *Phytophthora infestans*

Manuel D. Ospina-Giraldo, Richard W. Jones. USDA/ARS Vegetable Laboratory, Beltsville, MD

Glucose 6 phosphate isomerase (GPI) plays a key role in both glycolysis and gluconeogenesis and GPI isozyme pattern is the most widely used approach to characterize isolates of *P. infestans*. In addition to its well-known catalytic activity, GPI is considered a neurotrophic factor. Recent studies on *Xanthomonas campestris* pv. *citri* indicate that GPI also plays a role in bacterial pathogenicity. Despite the crucial role of GPI in the studying of *P. infestans*, the gene encoding this enzyme has not been characterized yet. Furthermore, it has been suggested that *P. infestans* contains multiple copies of the GPI coding sequence but this hypothesis remains to be demonstrated. In an attempt to elucidate these questions, we have cloned and characterized the GPI gene in various isolates of *P. infestans* as well as in several species of the genus Phytophthora. The confirmed cDNA GPI sequence consists of 1,671 base pairs. The gene, which has no introns, encodes a protein of 556 amino acids with a molecular weight of 60.78 kD. Multiple different alleles were identified by cloning and sequencing and Southern analysis indicates certain *P. infestans* isolates carry several copies of the gene. Phylogenetic studies indicate that *P. infestans* GPI is most closely related to sequences from plant and protozoan origin.

Poster Author Index

This index will be in the program book.

Organism Index

This index will be in the Program book as well as online.

Gene Index

Keyword Index